

Exhibit 1



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(19) **United States**(12) **Patent Application Publication** (10) **Pub. No.: US 2007/0010499 A1**
Asai et al. (43) **Pub. Date: Jan. 11, 2007**(54) **MEDICINAL COMPOSITIONS CONTAINING
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Ube-shi (JP)(21) **Appl. No.: 11/520,168**(22) **Filed: Sep. 13, 2006****Related U.S. Application Data**(60) Division of application No. 10/600,266, filed on Jun.
20, 2003, which is a continuation of application No.
PCT/JP01/11201, filed on Dec. 20, 2001.(30) **Foreign Application Priority Data**

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Publication Classification(51) **Int. Cl.**
A61K 31/60 (2006.01)
A61K 31/4743 (2006.01)
(52) **U.S. Cl.** 514/165; 514/301(57) **ABSTRACT**A combination of 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine or a pharmaceutically acceptable salt thereof, and aspirin, which possess excellent inhibitory activity against platelet aggregation and thrombogenesis, and is useful for preventing or treating diseases caused by thrombus or embolus.

MEDICINAL COMPOSITIONS CONTAINING ASPIRIN

[0001] This is a Divisional Application of application U.S. Ser. No. 10/600,266 filed Jun. 20, 2003, pending, which is a Continuation Application of International Application No. PCT/JPO1/11201 filed Dec. 20, 2001, which is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] This invention relates to pharmaceutical compositions containing 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine or a pharmaceutically acceptable salt thereof, and aspirin, as active ingredients [particularly pharmaceutical compositions for prevention or treatment (particularly for treatment) of diseases caused by thrombus or embolus]; to the use of 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine or a pharmaceutically acceptable salt thereof and aspirin for the manufacture of pharmaceutical compositions for prevention or treatment (particularly for treatment) of diseases caused by thrombus or embolus; and to methods for the prevention or treatment (particularly to methods for the treatment) of diseases caused by thrombus or embolus by administration of an effective amount of 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine or a pharmaceutically acceptable salt thereof and aspirin to warm-blooded animals (particularly humans).

[0003] 2-Acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine has been described in the Japanese Patent Application Publication No. Hei 6-41139, and possesses potent inhibitory activity against platelet aggregation. Furthermore, aspirin is well known to have an inhibiting activity against platelet aggregation, although the activity is low. However, pharmaceutical compositions containing both compounds have not been known.

BRIEF DESCRIPTION OF THE INVENTION

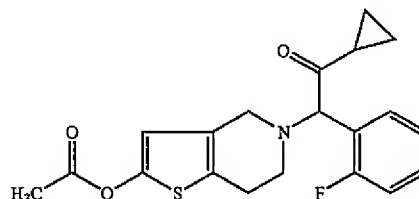
[0004] The present inventors have studied therapeutic agents with low toxicity that exert inhibitory activity against platelet aggregation and have found that the problems described above are solved by using pharmaceutical compositions comprising 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine or a pharmaceutically acceptable salt thereof and aspirin.

DETAILED DESCRIPTION OF THE INVENTION

[0005] The present invention provides pharmaceutical compositions containing 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine or a pharmaceutically acceptable salt thereof and aspirin as active ingredients [particularly pharmaceutical compositions for prevention or treatment (particularly for treatment) of diseases caused by thrombus or embolus]; the use of 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine or a pharmaceutically acceptable salt thereof, and aspirin, for the manufacture of pharmaceutical compositions [particularly pharmaceutical compositions for prevention or treatment (particularly for treatment) of diseases caused by thrombus or embolus]; and methods for the prevention or treatment

(particularly methods for treatment) of diseases caused by thrombus or embolus by administration of an effective amount of 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine or a pharmaceutically acceptable salt thereof, and aspirin, to warm-blooded animals (particularly humans), simultaneously or sequentially.

[0006] 2-Acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine, and pharmaceutically acceptable salts thereof, which is one of the active ingredients of the present invention, is a known compound. For instance, the compound has already been described in Japanese Patent Application Publication No. Hei 6-41139 and Japanese Patent Application Publication No. 2002-145882 (priority: Japanese Patent Application No. 2000-205539, and Japanese Patent Application No. 2000-266780). The chemical structure is described below.



[0007] The pharmaceutically acceptable salts of 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine may be, for example, hydrohalogenic acid salts such as hydrofluoride, hydrochloride, hydrobromide or hydroiodide; nitrate; perchlorate; sulfate; phosphate; C₁-C₄-alkanesulfonates optionally substituted by halogens such as methanesulfonate, trifluoromethanesulfonate, ethanesulfonate; C₆-C₁₀ arylsulfonates optionally substituted by C₁-C₄ alkyl groups such as benzenesulfonate or p-toluenesulfonate; C₁-C₆ aliphatic acid salts such as acetate, malate, fumarate, succinate, citrate, tartarate, oxalate or maleate; amino acid salts such as glycine salt, lysine salt, arginine salt, ornithine salt, glutamic acid salt or aspartic acid salt; and the preferred compounds are hydrohalogenates or C₁-C₆ aliphatic acid salts; and more preferred compounds are the hydrochloride or the maleate.

[0008] When one of the active ingredients of the present invention, 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine or a pharmaceutically acceptable salt thereof, is allowed to stand so that it is open to the atmosphere, it may become hydrated by absorption of water or adsorption of water. Such hydrated compounds are included in the present invention.

[0009] Further, one of the active ingredients of the present invention, 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine or a pharmaceutically acceptable salt thereof, may absorb some kinds of organic solvents and may form solvates in some cases, and these solvates are also included in the present invention.

[0010] Furthermore, since 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine has an asymmetric carbon atom, optical isomers exist based on the asymmetric carbon atom. These optical isomers are also included in the present invention.

[0011] The other active ingredient, aspirin, is a well-known compound, as an analgesic antipyretic.

[0012] The pharmaceutical compositions of the present invention (particularly pharmaceutical compositions for the prevention or treatment of diseases caused by thrombus or embolus) which contain 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine or a pharmaceutically acceptable salt thereof, and aspirin, as active ingredients, possess excellent inhibitory activity against platelet aggregation and thrombogenesis with short onset latency and low toxicity. Thus the pharmaceutical compositions of the present invention are useful as preventative or therapeutic agents (particularly as therapeutic agents) against diseases caused by thrombus or embolus, for example, diseases induced by platelet aggregation, including stable or unstable angina pectoris and so forth; cardiovascular or cerebrovascular disorders, e.g., thromboembolism, associated with atherosclerosis or diabetes mellitus, such as unstable angina pectoris, cerebral ischemic insult or restenosis due to angioplasty, endarterectomy or stent therapy; or thromboembolism caused by thromboembolization such as recurrent embolism after degradation of the original thrombus, embolism, ischemia-induced dementia, peripheral arteriopathy, thromboembolization associated with hemodialysis or atrial fibrillation, or thromboembolization in the vascular prosthesis, or in the bypass between the aorta and the coronary artery. Furthermore, the therapeutic agent of the present invention is administered to warm-blooded animals (particularly humans).

[0013] According to the present invention, the use in combination of 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine or a pharmaceutically acceptable salt thereof, and aspirin, results in more potent effectiveness than the use of each component alone. Furthermore, plasma levels of these agents do not have to be maintained at a certain level and higher during the same period, in order to produce their effects. It is believed that these 2 agents reach the receptors, at which they act in vivo, and turn on switches at the receptors to induce the effects. Even though the plasma level of one component of the pharmaceutical composition is too low to induce the effects with increasing time after the agent was administered, the switches at the receptors have already been turned on. Thus the preventative or therapeutic efficacy of the agent is expected by inhibiting thrombogenesis or embolization.

[0014] Therefore, when the other component of the pharmaceutical composition is administered later, the therapeutic effect of the compound administered later is expected to add to the therapeutic effects of the previously administered component. However, it is convenient clinically that both components are administered at the same time. Thus 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine or a pharmaceutically acceptable salt thereof and aspirin are simultaneously administered as a combination drug. In the case that both agents cannot be mixed technically, each component can be administered separately. Moreover, as described previously, since each component produces significant effects as a single form, each component can be sequentially administered at appropriate intervals. The maximum intervals between administration of each of the two components that can be accepted to elicit significant effects could be confirmed by clinical trials or animal studies.

[0015] The route for administration of 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine or a pharmaceutically acceptable salt thereof, and aspirin, which is employed in the present invention, is generally the oral route. However, other routes, for example, intravenous administration, can be used. Thus, the 2 components can be prepared respectively as separate formulations, or can be mixed physically to form a single formulation for administration. The single formulations of the mixed components are, for example, powders, granules, tablets, capsules and so forth, and can be prepared by regular formulation techniques, as described below.

[0016] These formulations are prepared by conventional methods by using excipients (organic excipients, for example, sugar derivatives such as lactose, sucrose, glucose, mannitol or sorbitol; starch derivatives such as corn starch, potato starch, α -starch or dextrin; cellulose derivatives such as crystalline cellulose; gum arabic; dextran; or pullulan; and inorganic excipients, for example, silicate derivatives such as light silicic acid anhydride, synthetic aluminum silicate, calcium silicate or magnesium aluminate metasilicate; phosphate derivatives such as calcium hydrogenphosphate; carbonates such as calcium carbonate; or sulfates such as calcium sulfate), lubricants (for example, stearic acid; metal stearate derivatives such as calcium stearate or magnesium stearate; talc; waxes such as beeswax or spermaceti; boric acid; adipic acid; sulfate derivatives such as sodium sulfate; glycol; fumaric acid; sodium benzoate; DL-leucine; lauryl sulfate derivatives such as sodium lauryl sulfate or magnesium lauryl sulfate; silicic acid derivatives such as silicic acid anhydride or silicic acid hydrate; and starch derivatives described above), binders (for example, hydroxypropyl cellulose, hydroxypropylmethylcellulose, poly(vinylpyrrolidone), polyethylene glycol and similar compounds described in the above excipients), disintegrators (for example, cellulose derivatives such as low substituted hydroxypropylcellulose, carboxymethylcellulose, calcium carboxymethylcellulose, internally cross-linked sodium carboxymethylcellulose; chemically modified starch/cellulose derivatives such as carboxymethylstarch, sodium carboxymethylstarch; cross-linked polyvinylpyrrolidone; or starch derivatives described above), emulsifiers (for example, colloidal clays such as bentonite or veegum; metal hydroxides such as magnesium hydroxide or aluminum hydroxide; anionic surfactants such as sodium lauryl sulfate or calcium stearate; cationic surfactants such as benzalkonium chloride; or nonionic surfactants such as polyoxyethylene alkyl ether, polyoxyethylenesorbitan ester of fatty acids or sucrose ester of fatty acids), stabilizers (for example, parahydroxybenzoates such as methylparaben or propylparaben; alcohols such as chlorobutanol, benzyl alcohol or phenylethyl alcohol; benzalkonium chlorides; phenol derivatives such as phenol or cresol; thimerosal; dehydroacetic acid; or sorbic acid), corrigents (for example, sweetening, souring and flavoring agents all of which are conventionally used), and diluents.

[0017] The dose and the dose ratio of 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine or pharmaceutically acceptable salt thereof, and aspirin, can be widely altered based on several factors such as activity of each compound, and the symptoms, age and body weight of the patients.

[0018] Generally, the lower limit of the oral dose (mg drug dose/time) is 0.1 mg (preferably, 1 mg) per time, while the upper limit is 1,000 mg (preferably, 500 mg) per time. The lower and upper limits of intravenous injection are 0.01 mg (preferably, 0.1 mg) and 500 mg (preferably, 250 mg), respectively. They are administered to the adult from 1 to 7 times a day based on the symptoms of the patient, simultaneously or sequentially.

[0019] Generally, the dose ratio of 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine or pharmaceutically acceptable salt thereof, and aspirin, is from 1:500 to 500:1 as their weight ratio.

EXAMPLES

[0020] The present invention is described in detail with examples and formulations in the following. However, the claim of the present invention is not restricted to the following description.

Example 1

[0021] Inhibitory Activity against Thrombogenesis

[0022] As the test animals, male Sprague Dawley rats of 7 weeks old were purchased from SLC Japan and 6 rats per group were used.

[0023] 2-Acetoxy-5-(α -cyclopropylcarbonyl)-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine was synthesized according to the method described in the Specification of Japanese Patent Application Publication No. Hei 6-41139 and was used, while aspirin was purchased from Sigma Chemical Co. and was used. Both compounds were suspended in 5% (w/v) gum arabic solution, and were diluted so as to be 1 ml/kg of administration volume and were orally administered.

[0024] The inhibitory activities of the compounds against thrombogenesis or thrombus formation were evaluated in the modified arterio-venous shunt thrombosis model in rats, which was described by Umetsu et al. [*Thromb. Haemost.*, 39, 74-83 (1978)].

[0025] The shunt tube was prepared as follows; i.e., both sides of a medical silicon tube of 12 cm length [inner diameter: 1.5 mm, outer diameter: 2.5 mm, purchased from KANEKA Medix Co., Ltd] were connected each to a polyethylene tube of 7 cm length [inner diameter: 0.5 mm, outer diameter: 1.0 mm, purchased from Natsume Seisakusho Co., Ltd.] covered with silicon via a medical silicon tube of 0.7 cm length [inner diameter: 1.0 mm, outer diameter: 1.5 mm, KANEKA Medix Co., Ltd] as connector. A surgical suture of 10 cm length was placed in the silicon tube of 12 cm length.

[0026] The animal was anesthetized with an intraperitoneal injection of 40 mg/kg of pentobarbital sodium (purchased from Abbott Laboratories Inc.), and the jugular of one side and the carotid of the other side were exposed. The arteriovenous shunt was made by cannulation of a shunt tube filled with heparin solution [30 units/kg, purchased from Fuso Pharmaceutical Co., Ltd] into the carotid and the jugular which had been previously exposed.

[0027] The test compounds were orally administered and the blood was started to circulate into the shunt area two hours after the administration. Thirty minutes after the

circulation was started, the shunt tube was removed, and the thrombus adsorbed on the surgical suture was weighed. The results are shown in Table 1. The results in the table are expressed as the average weight \pm SE (n=6).

TABLE 1

Compounds			
Compound A (mg/kg)	Aspirin (mg/kg)	Thrombus Weight (mg)	Inhibition Rate (%)
0	0	52.3 \pm 1.2	—
0	10	46.6 \pm 2.8	12.3 \pm 4.4
0.3	0	43.5 \pm 2.1	17.0 \pm 4.1
0.6	0	37.5 \pm 2.1	28.3 \pm 4.0
0.3	10	30.5 \pm 3.5	41.8 \pm 6.6
0.6	10	23.2 \pm 3.8	55.7 \pm 7.2

(Formulation 1)
Tablets

Compound A	10.0 mg
Aspirin	12.5 mg
Lactose	175.5 mg
Corn starch	50.0 mg
Magnesium stearate	2.0 mg
Total	250 mg

Compound A: 2-Acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine

[0028] The powders in the formula described in the above table are mixed, compressed with a tableting machine and formulated as a tablet containing 250 mg in total. The tablet can be coated with film or sugar, when necessary.

What is claimed is:

1. A method for the prevention of diseases caused by thrombus or embolus, comprising administering a pharmaceutical composition comprising 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine or a pharmaceutically acceptable salt thereof, and aspirin, as active ingredients, in their pharmacologically effective amounts, to a warm-blooded animal.

2. A method according to claim 1, in which the pharmaceutically acceptable salt is the hydrochloride or maleate.

3. A method according to claim 1 or claim 2, in which the warm-blooded animal is a human.

4. A method for the treatment of diseases caused by thrombus or embolus, comprising administering a pharmaceutical composition comprising 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine or a pharmaceutically acceptable salt thereof, and aspirin, as active ingredients, in their pharmacologically effective amounts, to a warm-blooded animal.

5. A method according to claim 4, in which the pharmaceutically acceptable salt is the hydrochloride or maleate.

6. A method according to claim 4 or claim 5, in which the warm-blooded animal is a human.

7. A method for the treatment of a patient undergoing stenting, angioplasty, and/or to prevent restenosis comprising administering a pharmaceutical composition comprising 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine or a pharmaceutically acceptable salt thereof, and aspirin, as active ingredients, in

their pharmacologically effective amounts, to a warm-blooded animal.

8. A method according to claim 7, in which the pharmaceutically acceptable salt is the hydrochloride or maleate.

9. A method according to claim 7 or claim 8, in which the warm-blooded animal is a human.

* * * * *

Exhibit 2



US005989578A

United States Patent [19]

Bernat et al.

[11] **Patent Number:** 5,989,578[45] **Date of Patent:** Nov. 23, 1999

[54] **ASSOCIATIONS OF ACTIVE PRINCIPLES
CONTAINING CLOPIDOGREL AND AN
ANTITHROMBOTIC AGENT**

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[86] **PCT No.:** PCT/FR97/00296

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PCT Pub. Date: Aug. 21, 1997

[30] **Foreign Application Priority Data**

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424/436; 424/456; 424/465; 424/489; 514/165;
514/301

[58] **Field of Search** 424/489, 422,
424/434, 427, 436, 456, 465; 514/165,
301

[56] **References Cited**

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[57] **ABSTRACT**

The invention relates to a pharmaceutical composition con-
taining an association of active principles, wherein the
active principles are clopidogrel and aspirin, each constitu-
ent being present in a free form or in the form of a
pharmaceutically acceptable salt.

25 Claims, No Drawings

ASSOCIATIONS OF ACTIVE PRINCIPLES CONTAINING CLOPIDOGREL AND AN ANTITHROMBOTIC AGENT

The subject of the present invention is a new combination of active ingredients with anti-platelet aggregation activity consisting of clopidogrel and aspirin, and pharmaceutical compositions containing them.

The active ingredients constituting the combination are present in the free state or in the form of one of their pharmacologically acceptable salts.

During the last decade, there has been a lot of interest in the study of the role played by the platelets in the development of diseases associated with atherosclerosis (myocardial infarction, angor, cerebral attack, peripheral arterial diseases and the like). The well-established role of the platelets in arterial thrombosis has allowed the development of numerous medicaments which inhibit the functions of the platelets and the discovery of the essential role of ADP in the thrombotic process has led to the development of ticlopidin, a potent antithrombotic agent. This thieno[3,2-c]pyridine derivative is described in Patent FR 73 03503. Ticlopidin selectively inhibits the platelet aggregation induced by ADP as well as that of other agonists, mediated by ADP [Féliste et al., *Thromb. Res.*, 1987, 48, 403-415].

In multicentre double-blind clinical studies, ticlopidin proved to be significantly more effective than aspirin or a placebo in the prevention of cerebral attack in patients having a high risk of vascular accidents (Gent et al., *Lancet*, 1989, 8649, 1215-1220; Hass et al., *N. Eng. J. Med.*, 1989, 321, 501-507). It also proved significantly more effective than the placebo in patients exhibiting a high risk of central or peripheral vascular accidents (Janzon et al., *Scand. J. Int. Med.*, 1990, 227, 301-308).

Although it is known, to date, that aspirin and ticlopidin act via two different mechanisms of action, numerous studies have compared the efficacy of these two medicaments and it is only very recently that a few studies have suggested that ticlopidin, administered in combination with aspirin, could be of great interest in relation to acute thrombosis, as a replacement of current poorly effective treatments, in patients in whom a metallic endovascular prostheses had been implanted (Van Belle et al., *Cor. Art. Dis.*, 1995, 6, 341-345).

The combination of ticlopidin and aspirin is claimed in patent FR 75 12084 for its use as anti-platelet aggregation agent endowed with a haemodynamic effect which is considerably qualitatively and quantitatively superior to that of ticlopidin alone. These results were demonstrated with the aid of pharmacological studies which related to the platelet aggregation inhibiting properties by making measurements of the platelet aggregation induced by ADP or collagen. The results which were obtained are predictive of a therapeutic importance of the ticlopidin-aspirin combination in some types of acute thrombosis following in particular some surgical operations but are not sufficient to deduce therefrom an indication in the secondary prevention of vascular accidents in atheromatous disease or alternatively in endarterectomy or fitting of metallic endovascular prostheses.

It is moreover known that other combinations of anti-platelet aggregation agents, such as for example the combination aspirin-dipyridamole, have been the subject of clinical studies against dipyridamole alone or aspirin alone in the study of the prevention of cerebral vascular accident or of occlusion of the vascular shunt in patients. The conclusion of these studies was that the aspirin-dipyridamole combination does not possess any significant

beneficial effect greater than that observed with dipyridamole alone or aspirin alone in the secondary prevention of cerebral atherothrombotic ischaemia or towards thrombosis (*Acta. Neurol. Scand.*, 1987, 76(6), 413-421; Thrombosis, 1994, Alert No. 12; Thrombosis, 1994, Alert No. 9. Thrombosis, 1993, Alert No. 9; Thrombosis, 1993, Alert No. 2).

The fitting of metallic endovascular prostheses at the coronary and carotid level can be considered today as an important therapeutic advance in the prevention and treatment of central and peripheral vascular accidents. However, these prostheses exhibit a potent prothrombotic effect due to their metallic nature which it is essential today to prevent with the aid of antithrombotic agents and mainly anti-platelet aggregation agents.

Another thienopyridin derivative, clopidogrel described in EP 099 802 has also proved to be a potent antithrombotic, acting through a mechanism of action identical to that of ticlopidin (Savi et al., *J. Pharmacol. Exp. Ther.*, 1994, 269, 772-777; Herbert et al., *Cardiovasc. Drug Rev.*, 1993, 11, 180-198).

Its use could be beneficial in relation to pathological states such as disorders of the cardiovascular and cerebrovascular system such as the thromboembolic disorders associated with atherosclerosis or with diabetes such as unstable angina, cerebral attack, restenosis following angioplasty, endarterectomy or fitting of metallic endovascular prostheses, with rethrombosis following thrombolysis, with infarction, with dementia of ischaemic origin, with peripheral arterial diseases, with haemodialyses, with auricular fibrillations or during the use of vascular prostheses or aortocoronary bypasses or in relation to stable or unstable angor.

Clopidogrel is, depending on the aggregation agents used, in animals and in man about 10 to 50 times more effective than ticlopidin. Furthermore, unlike the latter, clopidogrel exhibits a practically immediate anti-aggregation activity which appears within 15 minutes after the administration whereas ticlopidin requires, in order to be effective, a prolonged administration of at least 3 days at much higher doses. Furthermore, unlike ticlopidin, clopidogrel can be administered by the intravenous route and exhibits, by this route, anti-aggregation effects which are completely equivalent to those obtained by the oral route (Herbert et al., *Cardiovasc. Drug Rev.*, 1993, 11, 180-198). This is not the case for ticlopidin which can only be administered by the oral route.

Quite surprisingly and unexpectedly, the clopidogrel-aspirin combination of the invention proved to be endowed with a synergistic activity of the two active ingredients. This effect is characterized in relation to the aggregation of rabbit platelets with collagen, sole aggregation agent which can be used because of its joint dependency, by ADP and by the metabolism of arachidonic acid.

Furthermore, a similar synergistic effect was observed in relation to the formation of a thrombus of arterial origin induced by the implantation of a thrombogenic surface (silk thread) implanted in a catheter joining the carotid artery and the jugular vein of the rabbit.

The combinations according to the invention do not increase the haemorrhagic risk assessed by the extension of the bleeding time and are, moreover, not very toxic. Their toxicity is compatible with their use as medicament for the treatment of the disorders and of the diseases of thrombotic origin mentioned above.

The combinations according to the invention can be formulated in pharmaceutical compositions for administra-

tion to mammals, including man, for the treatment of the abovementioned diseases.

According to the invention, clopidogrel and aspirin can be administered in the form of a pharmaceutically acceptable salt.

These salts are those commonly used in pharmacy, such as acetate, benzoate, fumarate, maleate, citrate, tartrate, gentisate, methane sulphonate, ethane sulphonate, benzene sulphonate, lauryl sulphonate, dobesilate and paratoluene sulphonate.

In the text which follows, the quantities of clopidogrel and of aspirin are expressed as clopidogrel and aspirin equivalents in non-salified, free form.

Advantageously, the compositions of the invention comprise clopidogrel and aspirin in a molar ratio (aspirin/clopidogrel) of between 2.5 and 11.5, preferably between 5 and 9, better still between 7 and 8.

The combinations according to the invention can be used at daily doses of clopidogrel or of aspirin of 0.1 to 100 mg per kilo of body weight of the mammal to be treated.

In human beings, the dose may vary for each of the components from 1 to 500 mg per day, depending on the age of the subject to be treated and the type of treatment: prophylactic or curative.

In the pharmaceutical compositions of the present invention, the active ingredients are generally formulated in dosage units containing from 0.1 to 500 mg of the said active ingredient per unit dosage.

The subject of the present invention is therefore the pharmaceutical compositions which contain, as active ingredient, a combination of clopidogrel and aspirin. These compositions are preferably made so as to be administerable by the oral or parenteral route.

In the pharmaceutical compositions of the present invention for oral, sublingual, subcutaneous, intramuscular, intravenous, intradermal, local or rectal administration, the active ingredient may be administered in unit forms for administration, mixed with conventional pharmaceutical carriers, to animals and to human beings. The appropriate unit forms for administration comprise the forms for oral administration such as tablets, gelatin capsules, powders, granules and oral solutions or suspensions, the forms for sublingual or buccal administration, the forms for subcutaneous, intramuscular, intravenous, intranasal or intraocular administration and the forms for rectal administration.

When a solid composition in the form of tablets is prepared, the main active ingredient is mixed with a pharmaceutical vehicle such as gelatin, starch, lactose, magnesium stearate, talc, gum arabic and the like. The tablets can be coated with sucrose or other appropriate materials or alternatively they can be treated such that they have a prolonged or delayed activity and that they continuously liberate a predetermined quantity of active ingredient.

A preparation in the form of gelatin capsules is obtained by mixing the active ingredient with a diluent and by pouring the mixture obtained into soft or hard gelatin capsules.

A preparation in syrup or elixir form may contain the active ingredient together with a sweetener, preferably calorie free, methylparaben or propylparaben as antiseptic, as well as a taste enhancer and an appropriate colouring.

The water-dispersible granules or powders may contain the active ingredient mixed with dispersing agents or wetting agents, or suspending agents, such as polyvinylpyrrolidone, as well as sweeteners or flavour correctors.

For rectal administration, suppositories are used which are prepared with binders which melt at the rectal temperature, for example cocoa butter or polyethylene glycols.

For parenteral, intranasal or intraocular administration, aqueous suspensions, isotonic saline solutions, sterile and injectable solutions are used which contain dispersing agents and/or wetting agents which are pharmacologically compatible, for example propylene glycol or butylene glycol.

The active ingredient can also be formulated in the form of microcapsules, optionally with one or more carriers or additives.

The active ingredients of the combinations can also be provided in the form of a complex with cyclodextrin, for example α -, β - or γ -cyclodextrin, 2-hydroxypropyl- β -cyclodextrin or methyl- β -cyclodextrin.

When the compositions of the invention are administered in man by the parenteral or oral route, it is preferable that the daily dose of clopidogrel is between 50 and 100 mg, the daily dose of aspirin being between 100 and 500 mg.

It will be noted that according to the invention, clopidogrel and aspirin can both be administered by the oral route, or both by the parenteral route or one can be administered by the oral route (preferably aspirin) and the other by the parenteral route (preferably clopidogrel).

According to a preferred embodiment, the daily dose of clopidogrel administered in man by the parenteral and/or oral route is between 65 and 100 mg, better still between 65 and 85 mg, the daily dose of aspirin administered by the parenteral route being between 200 and 400 mg, better still between 315 and 335 mg.

Preferably, the dose of clopidogrel is in this case 75 mg per day and the dose of aspirin is 325 mg per day.

The combinations of active ingredients according to the invention have been the subject of pharmacological studies. Tests were carried out in relation to the test of aggregation of rabbit platelets with collagen as described above (Born et al., *J. Physiol.*, 1963, 168, 178-95). Briefly, 2.5 to 3 kg New Zealand rabbits were treated by the oral route with ticlopidin (100 mg/kg/d) for 3 days or by the intravenous route with clopidogrel (10 mg/kg). One hour after the last administration, the animals were treated by the intravenous route with aspirin (1 mg/kg).

Five minutes after the administration of aspirin, the animals were anaesthetized with ether and 2 ml of blood were collected from the median artery of the ear and mixed with 0.2 ml of a 3.8% solution of sodium citrate in water. The platelet-rich plasma was obtained by centrifugation of the blood at 500 g for 10 minutes at 15° C. The number of platelets was then adjusted to 10^6 cells per μ l with the aid of plasma low in platelets, obtained by centrifugation (3000 g, 15 min.) of anticoagulated blood.

The aggregation of the platelets was measured according to the method of Born (Born et al., *J. Physiol.*, 1963, 168, 178-95) with the aid of a double canal aggregometer (Chrono Log) with stirring (900 rpm) at 37° C. The aggregation of the platelets was induced by collagen (12.5 μ g/ml).

The antithrombotic effect of the clopidogrel or ticlopidin combination with aspirin was determined in relation to the formation of a thrombus on a silk thread present in an arteriovenous shunt implanted between the carotid artery and the jugular vein of the rabbit as described by Umetsu et al. (*Thromb. Haemostas.*, 1978, 39, 74-83). Briefly, 2.5 to 3 kg New Zealand rabbits were treated by the oral route with ticlopidin (100 mg/kg/d) for 3 days or by the intravenous route with clopidogrel (10 mg/kg).

The animals were anaesthetized by subcutaneous administration of sodium pentobarbital (30 mg/kg). Two polyethylene tubes 12 cm long (internal diameter: 0.6 mm; external diameter: 0.9 mm) attached by a central part 6 cm long (internal diameter: 0.9 mm) containing a silk thread 5 cm long were placed between the right carotid artery and the left jugular vein. One hour after the last administration of ticlopidin or of clopidogrel, the animals were treated by the intravenous route with aspirin (1 mg/kg). The central part of the shunt was then placed and then removed after 20 minutes of circulation of blood in the shunt. The weight of the thrombus present on the silk thread was then determined.

The results shown in TABLE 1 indicate that clopidogrel (10 mg/kg) or aspirin (1 mg/kg) administered by the intravenous route in a single dose in rabbit inhibit the aggregation of the platelets which is induced by collagen. Ticlopidin, administered by the oral route (100 mg/kg/d) for 3 days also exhibits a significant inhibitory effect in relation to the aggregation of the platelets with collagen.

In all cases, the joint administration of clopidogrel and aspirin resulted in a significant synergistic effect in relation to the aggregation of the platelets with collagen. That is to say that when the products were administered in combination, the anti-aggregation effect obtained was always greater than the mere sum of the effects of the two test products taken separately.

Compared with the mere additive effect observed between the anti-aggregation effect of ticlopidin and aspirin obtained and claimed in patent FR 73 03503, this activity is completely new and unexpected.

In the same manner, the antithrombotic activity of clopidogrel was potentiated by combination with aspirin. Under these conditions, and as in relation to the aggregation of the platelets with collagen, a significant synergistic effect was observed (TABLE 2).

TABLE 1

Effect of the products alone or in combination in relation to the aggregation of rabbit platelets with collagen.		
Active ingredients	Doses	% inhibition
Ticlopidin	100 mg/kg/D - 3 D	35 ± 3%
Clopidogrel	10 mg/kg	42 ± 6%
Aspirin	1 mg/kg	21 ± 2%
Ticlopidin + Aspirin	100 + 1 mg/kg	52 ± 6%
Clopidogrel + Aspirin	10 + 1 mg/kg	98 ± 1%

The values indicated in the table are the mean values on five experiments ± standard errors (n=5)

TABLE 2

Effect of the products alone or in combination in relation to the formation of an arterial thrombus on a silk thread implanted in an arteriovenous shunt in rabbit.		
Active ingredients	Doses	% inhibition
Ticlopidin	100 mg/kg/D - 3 D	25 ± 9%
Clopidogrel	10 mg/kg	34 ± 4%
Aspirin	1 mg/kg	19 ± 5%
Ticlopidin + Aspirin	100 + 1 mg/kg	45 ± 3%
Clopidogrel + Aspirin	10 + 1 mg/kg	82 ± 1%

The values indicated in the table are the mean values on five experiments ± standard errors (n=5)

We claim:

1. A pharmaceutical composition comprising a combination of clopidogrel aspirin, both constituents being present in the free state or in the form of a pharmaceutically acceptable salt.

2. A pharmaceutical composition according to claim 1, comprising clopidogrel and aspirin in combination with at least one pharmaceutical excipient.

3. A pharmaceutical composition according to claim 2 in a form administerable by the parenteral route or by the oral route.

4. A pharmaceutical composition according to claim 3 wherein clopidogrel and aspirin are present in an aspirin/clopidogrel molar ratio of between 2.5 and 11.5.

5. A pharmaceutical composition according to claim 1 for the treatment of a pathology induced by platelet aggregation.

6. A method for the treatment of a pathology induced by platelet aggregation, which comprises administering to a human in need of such treatment, a dose of 1 to 500 mg per day of clopidogrel and a dose of 1 to 500 mg per day of aspirin, the doses being expressed in equivalent quantity of clopidogrel and of aspirin in free form.

7. A method according to claim 6, in which the treatment involves the administration by the parenteral and/or oral route of 50 to 100 mg of clopidogrel per day and of 100 to 500 mg of aspirin per day.

8. A method according to claim 6, in which the treatment involves the administration by the parenteral and/or oral route of 65 to 100 mg of clopidogrel per day and of 200 to 400 mg of aspirin per day.

9. A method for the treatment of a pathology induced by platelet aggregation comprising the administration of an effective quantity of clopidogrel and, concomitantly, the administration of an effective quantity of aspirin, the clopidogrel and the aspirin being administered in the free state or in the form of a pharmaceutically acceptable salt.

10. A method according to claim 9, wherein the pathology induced by platelet aggregation is chosen from stable angor, unstable angor, disorders of the cardiovascular and cerebrovascular system, disorders associated with the use of vascular prostheses and disorders associated with aortic coronary bypasses.

11. A method according to claim 10, wherein the disorders of the cardiovascular and cerebrovascular system are chosen from thromboembolic disorders associated with atherosclerosis, with diabetes, with rethrombosis following thrombolysis, with infarction, with dementia of ischaemic origin, with peripheral arterial diseases, with haemodialysis and with auricular fibrillations.

12. A method according to claim 11, wherein the thromboembolic disorders associated with atherosclerosis and with diabetes are chosen from unstable angina, cerebral attack, restenosis following angioplasty, endarterectomy and the fitting of metallic endovascular prostheses.

13. A method according to claim 9, involving the administration in man of 1 to 500 mg per day of clopidogrel and of 1 to 500 mg per day of aspirin, the doses being expressed in equivalent quantity of clopidogrel and of aspirin in free form.

14. A method according to claim 9, involving the administration in man by the parenteral and/or oral route of 50 to 100 mg per day of clopidogrel and of 100 to 500 mg per day of aspirin, the doses being expressed in equivalent quantity of clopidogrel and of aspirin in free form.

15. A method according to claim 9, involving the administration in man by the parenteral and/or oral route of 65 to 100 mg per day of clopidogrel and of 200 to 400 mg per day of aspirin, the doses being expressed in equivalent quantity of clopidogrel and of aspirin in free form.

16. A method according to claim 15 involving the administration in man by the parenteral and/or oral route of 65 to 85 mg per day of clopidogrel and of 315 to 335 mg per day

of aspirin, the doses being expressed in equivalent quantity of clopidogrel and of aspirin in free form.

17. A pharmaceutical composition according to claim 4 wherein clopidogrel and aspirin are present in an aspirin/clopidogrel molar ratio of between 5 and 9.

18. A pharmaceutical composition according to claim 2 for the treatment of a pathology induced by platelet aggregation.

19. A pharmaceutical composition according to claim 3 for the treatment of a pathology induced by platelet aggregation.

20. A pharmaceutical composition according to claim 4 for the treatment of a pathology induced by platelet aggregation.

21. A pharmaceutical composition according to claim 17 for the treatment of a pathology induced by platelet aggregation.

22. A method according to claim 8, in which the treatment involves the administration by the parenteral and/or oral route of 65 to 85 mg of clopidogrel per day and of 315 to 335 mg of aspirin per day.

23. A method according to claim 6 wherein the pathology induced by platelet aggregation is chosen from stable angor, unstable angor, disorders of the cardiovascular and cerebrovascular system, disorders associated with aortocoronary bypasses and disorders associated with the use of vascular prostheses.

24. A method according to claim 23, wherein the disorders of the cardiovascular and cerebrovascular system are chosen from thromboembolic disorders associated with atherosclerosis or diabetes, rethrombosis following thrombolysis, infarction, dementia of ischaemic origin, peripheral arterial diseases, haemodialyses and auricular fibrillations.

25. A method according to claim 24, wherein the thromboembolic disorders associated with atherosclerosis or diabetes are chosen from unstable angina, cerebral attack, restenosis following angioplasty, endarterectomy, and the fitting of metallic endovascular prostheses.

* * * * *

Exhibit 3

CS-747, a New Platelet ADP Receptor Antagonist

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新規血小板ADP受容体拮抗薬CS-747

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I. Preface

Platelet activation and subsequent platelet aggregation play a central role in coronary artery diseases such as myocardial infarction and unstable angina, and cerebrovascular diseases such as stroke and transient ischemic attack.^{1,2)} Although platelets are activated by a variety of endogenous agonists, ADP is the earliest described and the most important platelet aggregation agonist. ADP induces primary aggregation.^{3,4)} In addition, ADP released from aggregating platelets induces secondary aggregation *via* the feedback process amplifying and propagating platelet activation induced by other agonists.^{5,6)} ADP activates platelets *via* ADP receptors, which have been tentatively designated as P2T receptors.^{6,7)} The P2T receptors are probably composed of three distinct receptors: the P2X₁, the ligand-gated ion channel, and two distinct G-protein-coupled ADP receptors (a G_q-linked P2Y₁ receptor and a G_i-linked P2T_{AC} receptor).⁸⁾

The importance of ADP in the pathogenesis of arterial thrombosis is supported by the recent demonstration that ADP-specific inhibitors of thienopyridine derivatives, such as ticlopidine and clopidogrel, are effective in reducing the risk of atherosclerotic vascular disease.^{9,10)} However, these drugs are by no means satisfactory in terms of their adverse effects and efficacy. Ticlopidine can have significant adverse effects at common dosage levels.¹¹⁾ Indeed, the use of ticlopidine is discouraged in patients with severe organ failure, and the blood cell count should be moni-

tored regularly during the first 3 months of ticlopidine administration because 1% of patients receiving ticlopidine may experience severe agranulocytosis.⁹⁾ Clopidogrel has been demonstrated to be safer than aspirin, the gold standard of antiplatelet agents at present. However, the clinical efficacy of clopidogrel over aspirin has proven just marginal.¹⁰⁾

The above-mentioned research and our interest to develop a novel antithrombotic agent prompted us to search for a more active antiplatelet drug with ADP-specific action, but with fewer side effects compared to ticlopidine and clopidogrel. In 1989, we began a collaborative study with Ube Industries (Yamaguchi, Japan). We performed *in vivo* screening tests on many compounds synthesized in Ube Industries. Among these compounds, CS-747 (2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine) was finally selected (Fig. 1) for further development. CS-747 was found to be an orally effective platelet aggregation inhibitor with high potency, fast onset and long duration of action. For example, CS-747 was 100-times more potent than ticlopidine in various experimental animals. Antiaggregatory effects of CS-747 were evident as early as 0.5 hr post-dose, and maintained up to 72 hr in

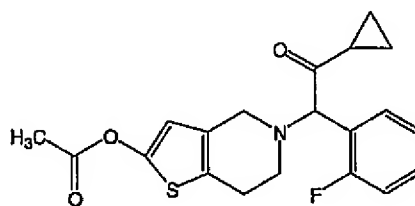


Fig. 1. Chemical structure of CS-747

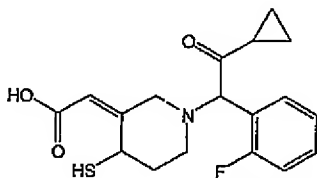


Fig. 2. Chemical structure of R-99224, an active metabolite of CS-747

animals. In contrast to this high pharmacological potency, CS-747 was revealed to exhibit minimum activity in general pharmacological and toxicological studies.

The metabolism and pharmacokinetics of CS-747 were examined in various animals. These studies revealed that CS-747 was rapidly and well absorbed after oral administration and extensively metabolized in the liver, most of it being excreted in the feces. CS-747, along with ticlopidine and clopidogrel,¹²⁾ was inactive *in vitro*, whereas these agents are all very active after oral administration. This raises the possibility of *in vivo* generation of active metabolites of thienopyridines, but they remain to be identified. R-99224 isolated from the incubation mixture of CS-747 and rat liver microsomes was found to show potent *in vitro* antiaggregatory activity. Its chemical structure (Fig. 2) was elucidated in 1994; R-99224 was detected in the blood of various animals that received oral administration of CS-747. The pharmacological profile of R-99224 matches that of CS-747, except for the high oral absorption of CS-747, with potent, selective and irreversible *in vitro* antagonistic activities against P2T_{AC} receptors. The results suggest that R-99224 is an active metabolite of CS-747 and contributes to

the pharmacological activities of CS-747 after its oral administration.

These pre-clinical studies on CS-747 have suggested its potential as a novel antiplatelet agent with a wide safety margin. In particular, comparative toxicologic studies with clopidogrel revealed that CS-747 was less toxic than clopidogrel. Thus, Phase I clinical studies were started in the U.K. in 1997. Single oral administration of CS-747 at 30 mg produced potent inhibition of platelet aggregation with a fast onset and a long duration in healthy volunteers. This is noteworthy, since maximal effects were observed only after multiple administrations of ticlopidine (500 mg/day)⁹⁾ and clopidogrel (75 mg/day).¹³⁾ A multiple dose of CS-747 administered once daily for 10 days was tolerable, and produced a significant and steady-state inhibition of platelet aggregation. There were no abnormal changes in the safety parameters in any dose groups.

In this review, we will examine the pre-clinical and clinical evidence that suggests the clinical usefulness of CS-747, a novel antiplatelet agent with P2T_{AC} antagonistic action, for patients with occlusive vascular diseases.

(Fumitoshi Asai)

II. Physicochemical Properties

The physicochemical properties of CS-747, including the appearance, melting point, solubility, partition coefficient, elemental analysis, spectroscopic properties, and acid dissociation constant, were investigated. The stability of CS-747 in

buffered solutions, and in the solid state, was also examined. To confirm the rationale for developing CS-747 as a racemic mixture, racemization and deacetylation reactions of CS-747 in aqueous solutions were studied.

1. Physicochemistry

1) Appearance

CS-747 occurs as a white to pale-yellow crystalline powder containing masses.

2) Melting point

The melting point of CS-747 is 121°C-122°C.

3) Solubility and partition coefficient

Solubility data of CS-747 in various organic solvents and aqueous solutions at 20°C are given in Table 1 and Table 2, respectively. CS-747 is freely soluble in benzene, chloroform, and acetone, and slightly soluble in methanol and ethanol; however, it is practically insoluble in water and neutral buffered solution. With decreasing pH, CS-747 becomes slightly soluble. It is assumed that the solubility of CS-747 in aqueous solution depends on its dissociation constant (pK_a) of 5.40 (see

Table 2. Solubility of CS-747 in aqueous solutions

pH (20°C)	Solubility (μg/mL)
3.57	59.8
4.38	37.5
4.85	14.2
5.25	5.54
5.47	5.82
6.00	4.01
7.02	0.08
7.12	0.15

Table 3. Elemental analysis (Batch No. 5)

Element	C	H	N	F	S
Found (%)	64.40	5.57	3.75	5.23	8.78
Calculated (%)	64.33	5.40	3.75	5.09	8.59

below 6) Dissociation constant). The partition coefficient ($\log P$) of CS-747 was determined to be 3.7 between n-octanol and phosphate buffer (pH 7.0) using the flask-shaking method.

4) Elemental analysis

The results of the elemental analysis are presented in Table 3. The data conformed to the calculated values (C, 64.33; H, 5.40; N, 3.75; F, 5.09; S, 8.59).

5) Spectrophotometric analysis

The UV spectrum of CS-747 in ethanol is presented in Fig. 1. It exhibited no absorption maximum in the range of 200 nm to 350 nm, and showed three shoulders at around 218, 238, and 258 nm. The corresponding molar extinction coefficients of these peaks were 1.28×10^4 , 7.68×10^3 , and $6.44 \times 10^3 \text{ cm}^2 \text{ mol}^{-1}$, respectively.

Table 1. Solubility of CS-747 in organic solvents

Solvent (20°C)	Solubility (mg/mL)
Benzene	>100
Chloroform	>100
Acetone	>100
Ethyl acetate	82.5
Acetonitrile	67.0
Methanol	9.01
Ethanol	5.91
n-Hexane	0.65

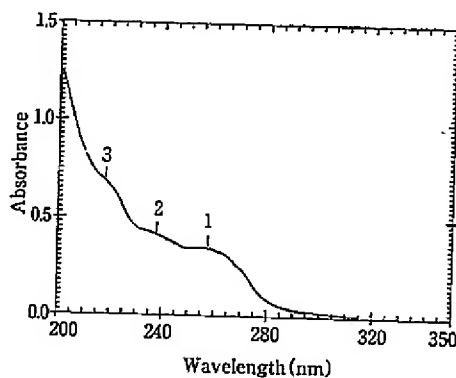


Fig. 1. UV spectrum of CS-747 in ethanol

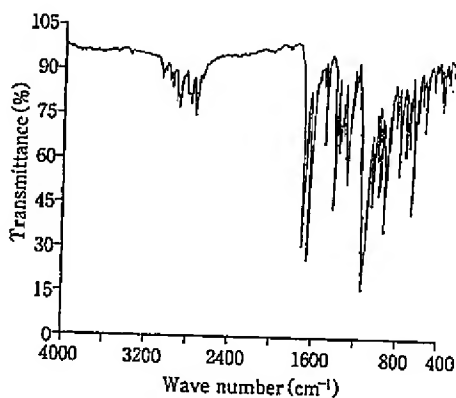


Fig. 2. IR spectrum of CS-747

The IR spectrum of CS-747, determined by the potassium bromide disc method, is shown in Fig. 2. The assignments of the characteristic absorption bands in the IR spectrum are listed in Table 4.

6) Dissociation constant

The pK_a for CS-747, determined spectrophotometrically, was found to be 5.40 ± 0.05 at 25°C . This pK_a is assumed to correspond to the protonation of the tertiary amine structure in the piperidine moiety.

Table 4. The IR band assignments for CS-747

Wave number (cm^{-1})	Assignment
3013, 3035	Aromatic C-H stretching
2718-2944	Aliphatic C-H stretching
1757	C=O stretching of ester
1703	C=O stretching of ketone
1489	C-H deformation
1193	C-O asymmetrical stretching
758	C-H out-of-plane deformation of the disubstituted benzene ring

Table 5. Stability of CS-747 in aqueous solutions at various pH levels

pH	1 hour	3 hours	6 hours	24 hours
1.2 (J.P.1)	76.6	74.9	71.5	33.7
2.0	76.7	73.4	71.0	59.6
4.0	95.7	94.7	91.7	77.6
6.8 (J.P.2)	98.5	88.1	81.4	49.0
8.0	93.3	80.7	67.0	29.0

The concentration of CS-747 was $100 \mu\text{g/mL}$ in 20% $\text{CH}_3\text{CN}/\text{buffer}$.

2. Stability

The stability of CS-747 in aqueous solutions was examined under various conditions from pH 1.2 to pH 8.0 at 37°C over a period of 24 hr. The results are summarized in Table 5. It was found that CS-747 is rather stable at pH 4, giving 78% as the residual percentage after 24 hr but it was unstable in solutions having a pH lower or higher than 4.

Long-term and accelerated storage tests of CS-747 in the solid state were performed, as shown in Table 6. CS-747 was confirmed to be stable at 25°C -60% RH (relative humidity) for 18 months, and at 40°C for 12 months when stored in closed glass bottles.

Table 6. Stability of CS-747 in the solid state

Storage conditions	Initial	1 month	3 months	6 months	9 months	12 months	18 months
25°C-60% RH	101.0	100.5	100.7	100.6	100.6	100.7	100.0
40°C	100.1	99.8	100.2	100.6	100.4	100.2	

Table 7. Photostability of CS-747 exposed to a cool white fluorescent lamp

Test	Initial	Lux-hours			Reference
		600000	1200000	2400000	
Assay (%)	100.0	99.3	98.9	98.3	99.8

Table 8. Photostability of CS-747 exposed to a near-ultraviolet lamp

Test	Initial	W-hr/m ²			Reference
		120	240	480	
Assay (%)	100.0	99.8	99.6	99.0	99.5

The photostability of CS-747 was tested under light exposure of a cool white fluorescent lamp and a near-ultraviolet lamp. The results are presented in Table 7 and Table 8, respectively. It was found that CS-747 is stable in both light exposures.

3. Racemization and Deacetylation Reactions of CS-747 in Aqueous Solution

CS-747 has one chiral center, and thus consists of two optical isomers, as shown in Fig. 3. The kinetic properties of racemization and deacetylation of each optical isomer were studied in aqueous solutions at various pHs at 37°C. The enantiomer ratios of CS-747 as a function of time, starting from the *R*- or *S*-form in pH 7.4 aqueous solution at 37°C, are shown in Fig. 4. The kinetic plots for the racemization are presented in Fig. 5. The rate con-

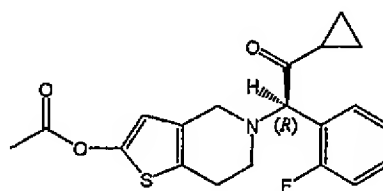
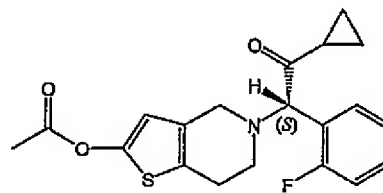
*R* form of CS-747*S* form of CS-747

Fig. 3. Chemical structures of optical isomers of CS-747

stants and half-lives ($T_{1/2}$) of the racemization are summarized in Table 9. The pH

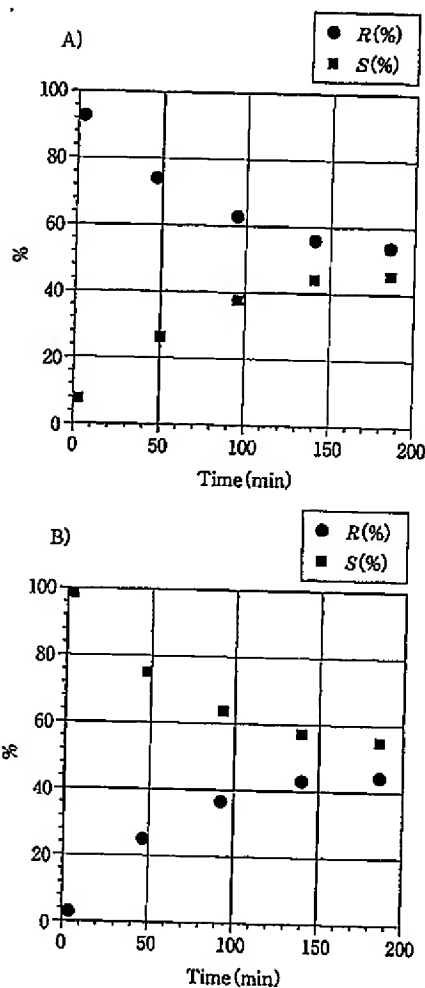


Fig. 4. Racemization of the R form and S form (A and B, respectively) in pH 7.4 aqueous solution at 37°C

dependence of the $T_{1/2}$ in the racemization of each optical isomer of CS-747 is shown in Fig. 6. The racemization occurred rapidly, with a $T_{1/2}$ of about 1 hr at pH of no lower than 4.

The deacetylation of CS-747 was also found to proceed similarly under the same

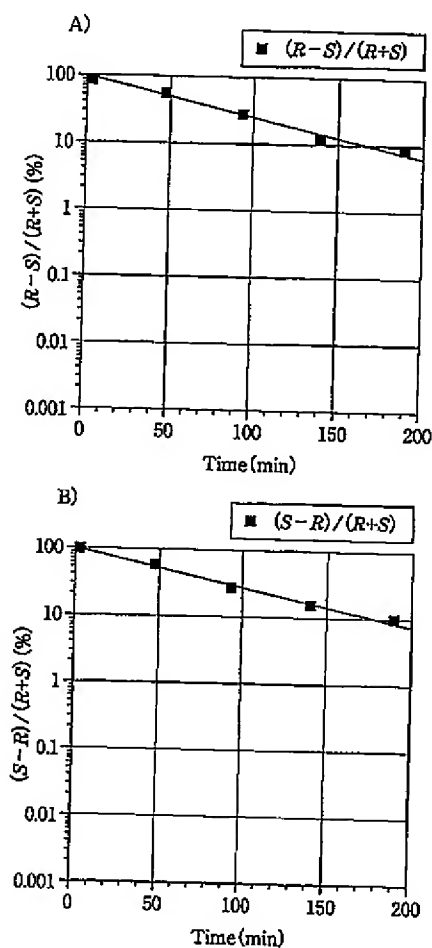


Fig. 5. Kinetic plots for the racemization of the R form and S form (A and B, respectively) in pH 7.4 aqueous solution at 37°C

conditions. The kinetic plots of the deacetylation of each optical isomer in pH 7.4 aqueous solution at 37°C are shown in Fig. 7. The rate constants and $T_{1/2}$ values for the deacetylation are summarized in Table 10. The pH dependence of $T_{1/2}$ in the deacetylation of each optical isomer of CS-747 is plotted in Fig. 8. The deacetyla-

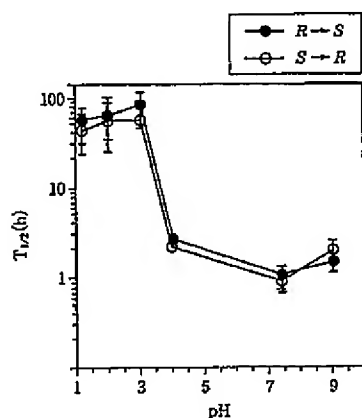


Fig. 6. $T_{1/2}$ for racemization of each optical isomer of CS-747 at 37°C with respect to the pH. Error bars indicate standard deviation (mean \pm S.D., $n=3$).

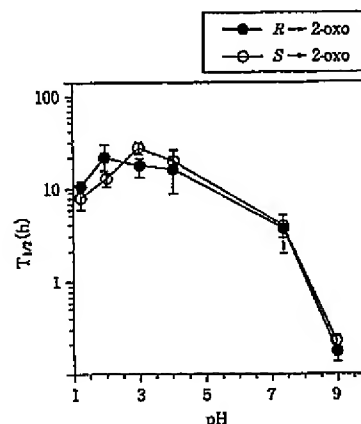


Fig. 8. $T_{1/2}$ for deacetylation of each optical isomer of CS-747 at 37°C with respect to the pH. Error bars indicate standard deviation (mean \pm S.D., $n=3$).

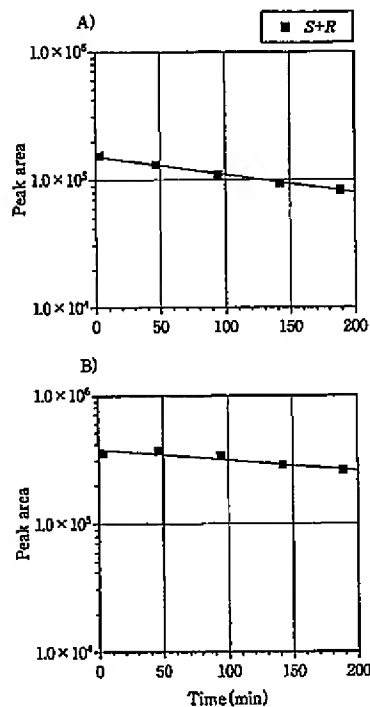


Fig. 7. Deacetylation of the R form and S form (A and B, respectively) in pH 7.4 aqueous solution at 37°C

tion reaction was found to be relatively slow, with a $T_{1/2}$ of over 3.9 hr, compared with the racemization.

It is predicted that each optical isomer of CS-747 will be stable under the acidic conditions of the stomach after oral administration. However, after reaching the intestinal tract, which is maintained at a neutral pH, the compound will rapidly undergo racemization before absorption. These data support the concept that the development of CS-747 as a single enantiomer has no rationale. The deacetylation reaction gives the 2-oxo form, which is the relatively stable intermediate leading to the pharmacologically active metabolite, as will be described later, and the reaction is considered to proceed enzymatically in the *in vivo* situation, rather than the non-enzymatic mechanism shown in this chapter.

(Tomonori Konse)

Table 9. Kinetic parameters of racemization of CS-747 optical isomers

pH	<i>R</i> → <i>S</i>		<i>S</i> → <i>R</i>	
	<i>k</i> (×10 ⁻² h ⁻¹)	<i>T</i> _{1/2} (h)	<i>k</i> (×10 ⁻² h ⁻¹)	<i>T</i> _{1/2} (h)
1.2 (J.P.1)	1.6 ± 0.4	54.7 ± 23.6	2.1 ± 1.3	43.1 ± 20.0
2.0	1.4 ± 0.7	62.0 ± 26.6	2.8 ± 3.3	56.6 ± 30.7
3.0	0.9 ± 0.4	83.0 ± 22.3	1.3 ± 0.1	53.5 ± 2.8
4.0	25.0 ± 2.7	2.8 ± 0.2	29.7 ± 1.2	2.3 ± 0.1
7.4	68.3 ± 24.6	1.1 ± 0.3	76.0 ± 21.0	1.0 ± 0.2
9.0	47.1 ± 12.6	1.5 ± 0.3	37.5 ± 9.7	2.0 ± 0.5

Table 10. Kinetic parameters of deacetylation of CS-747 optical isomers

pH	<i>R</i> → 2-oxo form		<i>S</i> → 2-oxo form	
	<i>k</i> (×10 ⁻² h ⁻¹)	<i>T</i> _{1/2} (h)	<i>k</i> (×10 ⁻² h ⁻¹)	<i>T</i> _{1/2} (h)
1.2 (J.P.1)	6.3 ± 0.7	11.1 ± 1.1	8.2 ± 1.3	8.6 ± 1.5
2.0	3.3 ± 0.9	33.4 ± 6.9	5.5 ± 0.7	12.8 ± 1.7
3.0	3.9 ± 0.6	17.9 ± 6.5	2.4 ± 0.07	28.8 ± 0.8
4.0	4.5 ± 1.8	17.1 ± 0.02	3.3 ± 0.7	21.4 ± 5.0
7.4	17.7 ± 1.2	3.9 ± 0.3	18.5 ± 8.2	4.2 ± 1.6
9.0	374.0 ± 42.2	0.2 ± 0.02	332.0 ± 43.5	0.2 ± 0.03

III. Pharmacology

1. Inhibitory Activity of CS-747 on Platelet Aggregation

1) Single oral administration

Time courses of the *ex vivo* effects of CS-747 (1–10 mg/kg, *p.o.*) and clopidogrel (10–100 mg/kg, *p.o.*) on ADP (3 μM)-induced platelet aggregation in platelet-rich plasma (PRP) were examined after a single administration of each agent to SD rats (Figs. 1 and 2). At 0.5 hr after dosing, more than 50% inhibition was observed in CS-747-treated SD rats, while clopidogrel had minimal effect, suggesting an early onset of the antiplatelet action of CS-747 (Fig. 1). Although the precise

mechanism responsible for the rapid onset of CS-747's effect remains to be elucidated, one possible explanation is that CS-747 may be more rapidly metabolized to its active metabolite *in vivo* (see ADME).

Maximum effects of both agents were observed 4 hr after the dosing in SD rats, but the effect of CS-747 was more potent than that of clopidogrel (Figs. 1 and 2). The inhibitory effects of CS-747 (1 and 3 mg/kg) and clopidogrel (10 and 30 mg/kg) were long-lasting, and were still significant 72 hr after dosing (Fig. 2). These similar effects of both agents had disappeared by 96 hr after dosing. The durations of inhibition of platelet aggregation by CS-747 and clopidogrel were comparable to the life span of circulating platelets

in the rat.^{14,15)}

2) Three-day oral administration

CS-747, clopidogrel, and ticlopidine were orally administered to SD rats once a day for 3 days. Blood was collected 4 hr after the final administration of the agents, and effects of the agents on ADP (0.3–30 μ M)-induced platelet aggregation were

measured using PRP. CS-747 (0.3–3 mg/kg/day, *p.o.*) inhibited platelet aggregation in a dose-dependent manner (Fig. 3). Clopidogrel (3–30 mg/kg/day, *p.o.*) also inhibited platelet aggregation, but the effect of clopidogrel was 10-fold less potent than that of CS-747. Ticlopidine (30–300 mg/kg/day, *p.o.*) showed minimal effect. CS-747, given orally, also showed

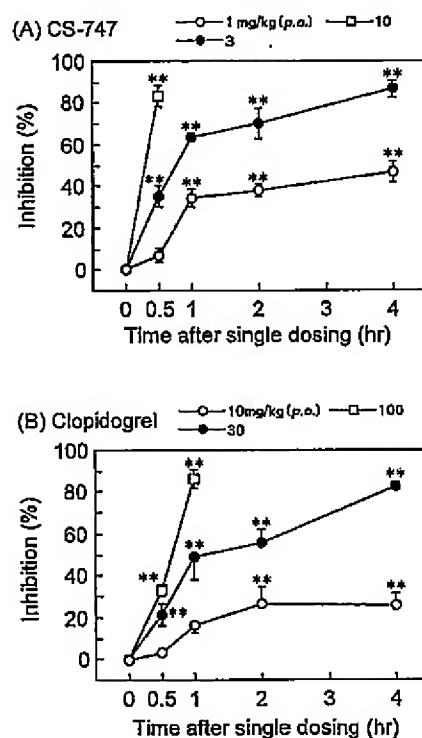


Fig. 1. Effects of single oral administrations of CS-747 (A) and clopidogrel (B) on platelet aggregation up to 4 hr post-dose in rats. ADP at a concentration of 3 μ M was used as the agonist in PRP aggregation. Results are represented as the mean \pm S.E.M. ($n=6$). ** $p<0.01$ vs. control (vehicle-treated group).

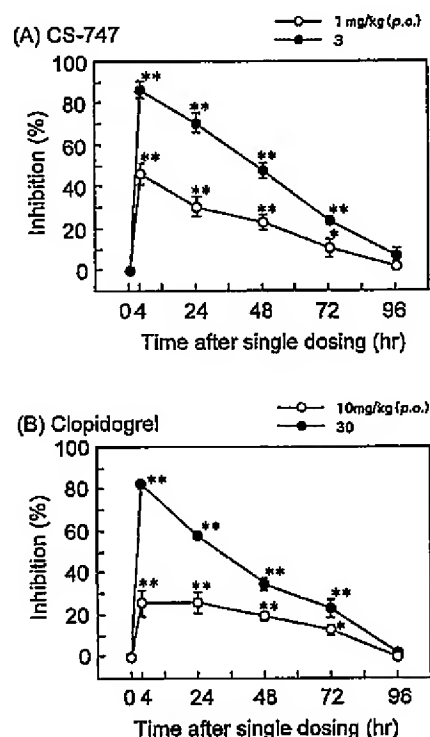


Fig. 2. Effects of single oral administrations of CS-747 (A) and clopidogrel (B) on platelet aggregation 4–96 hr post-dose in rats. ADP at a concentration of 3 μ M was used as the agonist in PRP aggregation. Results are represented as the mean \pm S.E.M. ($n=5-6$). * $p<0.05$, ** $p<0.01$ vs. control (vehicle-treated group).

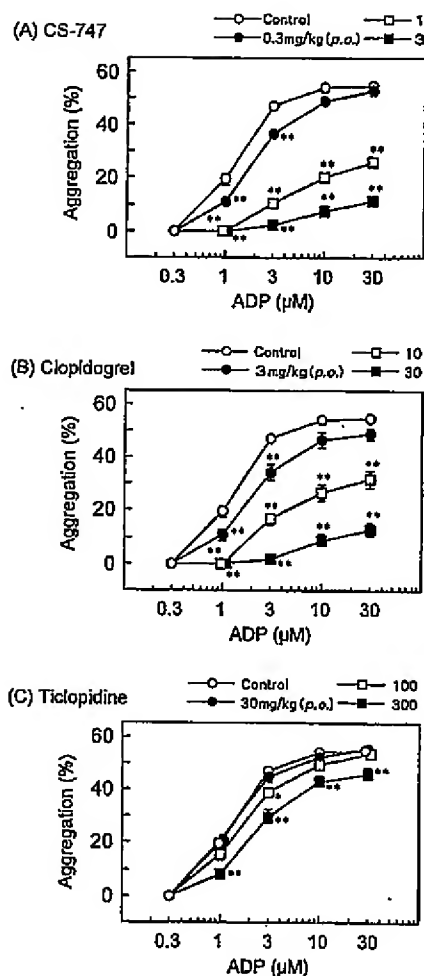


Fig. 3. *Ex vivo* effects of 3-day repeated administrations of CS-747 (A), clopidogrel (B), and ticlopidine (C) on ADP-induced platelet aggregation in rats. Agents were orally administered to rats once a day for 3 days. The aggregation in PRP was measured 4 hr after the final dosing. Results are represented as the mean \pm S.E.M. ($n=6$). * $p<0.05$, ** $p<0.01$ vs. control (vehicle-treated group).

more potent inhibition of platelet aggregation than clopidogrel and ticlopidine in Hartley guinea pigs, beagle dogs, and cynomolgus monkeys (data not shown). These results clearly show that CS-747 is a more potent antiplatelet agent in comparison with clopidogrel and ticlopidine.

3) Two-week oral administration

Inhibitory effects of CS-747, administered repeatedly over 2 weeks, on *ex vivo* platelet aggregation in cynomolgus monkeys were investigated. ADP (10 μ M)-induced platelet aggregation was measured on Days 0 (pre), 1, 3, 5, 7, 14, 17, 21, and 28 during the experimental period. CS-747 (0.1 and 0.3 mg/kg/day, *p.o.*) inhibited platelet aggregation in a dose-

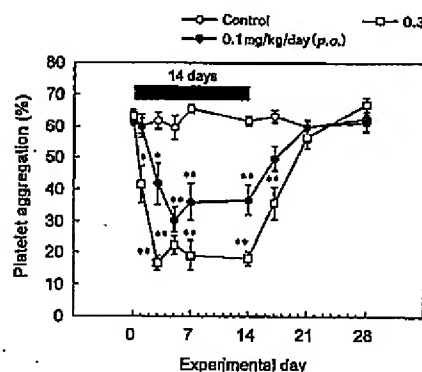


Fig. 4. Effects of repeated administrations of CS-747 on ADP (10 μ M)-induced platelet aggregation in cynomolgus monkeys. CS-747 was orally administered to monkeys once a day for 14 days. Platelet aggregation was measured 4 hr after dosing on each day. Data are represented as the mean \pm S.E.M. ($n=5$). * $p<0.05$, ** $p<0.01$ vs. control (vehicle-treated group).

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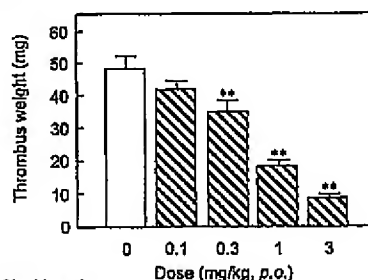
dependent manner (Fig. 4). Inhibitory effects of CS-747 (0.1 and 0.3 mg/kg/day) reached a plateau from Day 3 to 5. After completion of the CS-747 dosing period, platelet aggregation gradually returned to the pre-treatment level on Day 21 (7 days after the final dosing), suggesting long-lasting action of CS-747. In beagle dogs, CS-747 (0.03-0.3 mg/kg/day, *p.o.*) also showed a similar time course of antiplatelet action (data not shown). These data indicate that CS-747 has potent and long-lasting inhibitory effects on platelet aggregation.

2. Antithrombotic Effects of CS-747

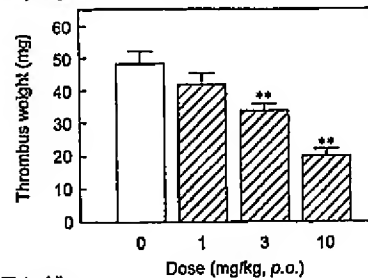
1) Arteriovenous shunt thrombosis model

Efficacy of CS-747 in an arteriovenous shunt thrombosis model, originally described by Umetsu and Sanai,¹⁶ was determined in SD rats (Fig. 5). Thrombus formation was assessed by weighing the wet weight of the thrombus formed in the shunt tube. CS-747 and clopidogrel were orally administered once to SD rats, while ticlopidine was orally administered once daily for 3 days. Blood circulation was restarted 4 hr after the single or the final dosing. In the control groups, the thrombus weights after 30 min of blood circulation were 48.8-55.1 mg ($n=7-10$). CS-747 (0.1-3 mg/kg, *p.o.*) prevented thrombus formation in a dose-dependent manner, with an ED_{50} value of 0.65 mg/kg. In contrast, clopidogrel and ticlopidine were less potent than CS-747 in this model; the ED_{50} values for clopidogrel and ticlopidine were 7.0 mg/kg and >300 mg/kg/day, respectively.

(A) CS-747



(B) Clopidogrel



(C) Ticlopidine

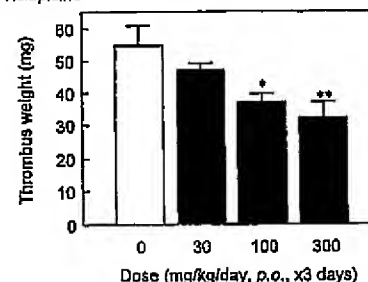


Fig. 5. Antithrombotic effects of CS-747 (A), clopidogrel (B), and ticlopidine (C) in an arteriovenous shunt thrombosis model in rats. CS-747 and clopidogrel were administered once orally to rats 4 hr before blood circulation. Ticlopidine was orally administered daily for 3 days to rats. Blood circulation in ticlopidine-treated rats was started 4 hr after the last dosing. Results are represented as the mean \pm S.E.M. ($n=7-10$). * $p<0.05$, ** $p<0.01$ vs. control (vehicle-treated group).

2) Photochemically induced arterial thrombosis (PIT) model

Table 1. Effects of CS-747, clopidogrel, and ticlopidine on a photochemically induced thrombosis model in rats

Treatment	Dose (mg/kg, <i>p.o.</i>)	N	Time to occlusion (min)	Incidence of non-occlusion
Control	—	10	6.80 ± 0.47	0/10
CS-747	0.3	8	9.33 ± 1.73	1/8
	1	8	10.03 ± 2.42	2/8
	3	8	13.54 ± 2.52*	4/8*
Clopidogrel	10	8	11.92 ± 2.81	3/8
Ticlopidine	100	8	6.89 ± 0.90	0/8

Experiments were performed 4 hr after *p.o.* administration of the agents. Data for time to occlusion are expressed as the mean ± S.E.M. **p*<0.05 vs. control.

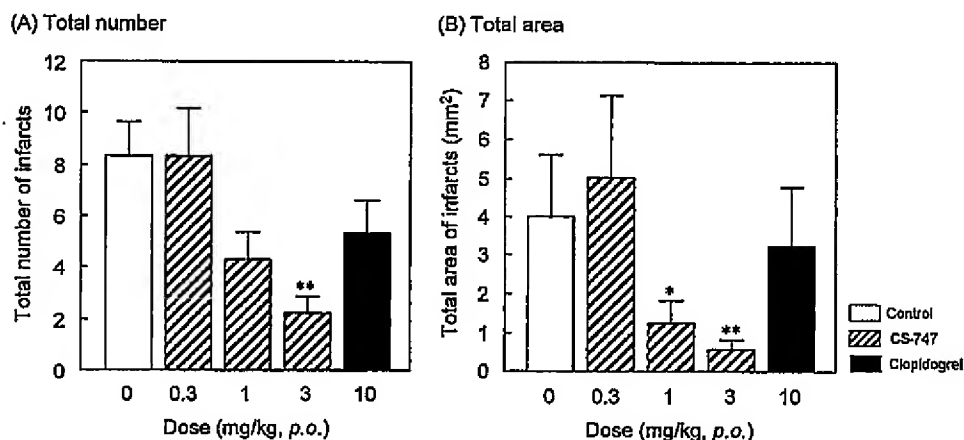


Fig. 6. Effects of CS-747 and clopidogrel on cerebral infarction in a rat PIT model. (A) Total number of infarcts and (B) total infarct area (mm²). Results are expressed as the mean ± S.E.M. (n=16). **p*<0.05, ***p*<0.01 vs. control.

We compared CS-747, clopidogrel, and ticlopidine for their antithrombotic effects in a femoral arterial thrombosis model in SD rats (Table 1). Thrombosis was induced in the femoral artery of anesthetized SD rats by endothelial damage due to the photochemical reaction between rose bengal (20 mg/kg, *i.v.*) transilluminated with green light (540 nm), according to the method by Takiguchi *et al.*,¹⁷⁾ with slight

modification. The agents, or vehicle, were orally administered 4 hr before the induction of thrombosis. Blood circulation in the artery was obstructed at 6.80 ± 0.47 min (n=10) after initiation of the photochemical reaction in the control rats. CS-747 prolonged the time to occlusion in a dose-dependent manner. A statistically significant (*p*<0.05) prolongation was observed at 3 mg/kg of CS-747 (13.54 ±

lel

2.52 min, $n=8$). Clopidogrel caused a mild prolongation of the time to occlusion, but it was not statistically significant ($p>0.05$). Ticlopidine (100 mg/kg, *p.o.*) had no effects in this model even when the agent was administered at a very high dose for 3 consecutive days.

3) Embolic cerebral infarction model

Effects of CS-747 were examined on embolic cerebral infarction in a rat model (Fig. 6). Four hours after oral administration of the agents, SD rats were anesthetized and the right common carotid artery was transilluminated with green light (wavelength: 540 nm); rose bengal (40 mg/kg, *i.v.*) was injected to induce a non-occlusive thrombus formation. SD rats were euthanized 24 hr later, and the brain was excised. Histological analysis showed that CS-747 (0.3-3 mg/kg, *p.o.*) reduced the total area of cerebral infarcts in a dose-dependent manner. Comparatively,

clopidogrel (10 mg/kg, *p.o.*) had similar, yet milder, effects on cerebral infarction, *i.e.* the agent was 10 times less potent than CS-747.

4) Summary of the effects of CS-747 in several experimental thrombosis models

Antithrombotic effects of CS-747 in several thrombosis models are summarized in Table 2. In addition to the arteriovenous shunt and PIT models in SD rats, CS-747 showed potent antithrombotic effects on electrically induced arterial thrombosis models, which have often been used to assess the efficacy of antithrombotic agents,^{18,19} in SD rats and Japanese white rabbits. The minimum effective dose in both of these animal models was 0.3 mg/kg (*p.o.*), suggesting potent antithrombotic effects by CS-747. CS-747 was also effective in peripheral artery occlusive diseases in Wistar rats and

Table 2. Summary of the antithrombotic effects of CS-747 on thrombosis models in experimental animals

Thrombosis model	Animal	Vessels	Minimum effective dose (mg/kg, <i>p.o.</i>)	Administration
Arteriovenous shunt model	Rat	Carotid artery and vein	0.3	Single
Photochemically-induced arterial thrombosis	Rat	Femoral artery	3	Single
Electrically-induced arterial thrombosis	Rat	Carotid artery	0.3	Single
	Rabbit	Femoral artery	0.3	Single
Peripheral artery occlusive model	Rat	Femoral artery	0.3	Multiple (11 days)
Embolic cerebral infarction model	Rat	Carotid artery	1	Single
Cholesterol-induced atherosclerosis model	Guinea pig	Aortic arch	3	Multiple (20 weeks)
	Rabbit	Abdominal aorta	3	Multiple (10 weeks)

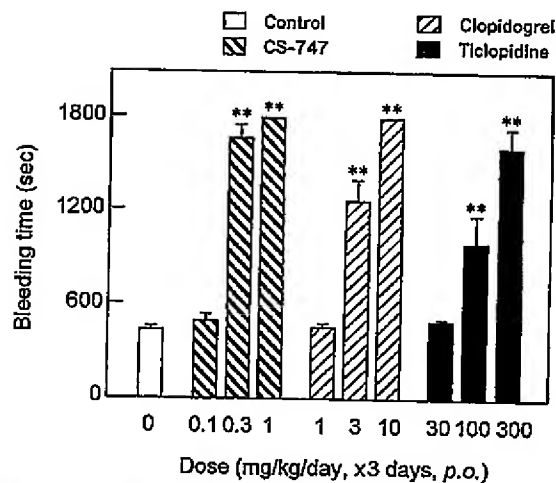


Fig. 7. Effects of 3-day repeated administrations of CS-747, clopidogrel, and ticlopidine on tail transection bleeding time in rats. Bleeding time was measured 4 hr after the final dosing. Results are expressed as the mean \pm S.E.M. ($n=7$). ** $p<0.01$ vs. control (vehicle-treated group).

embolic cerebral infarction models in SD rats. Moreover, CS-747 inhibited the development of atherosclerotic lesions in hypercholesterolemic Japanese white rabbits and Hartley guinea pigs, although the minimum effective dose (3 mg/kg/day, *p.o.*) was slightly higher. These results clearly demonstrate that CS-747 has potent antithrombotic activities on several thrombosis models, supporting the fact that CS-747 should be effective in several vascular thromboembolic disorders.

3. Effects of CS-747 on Bleeding Time

Effects of CS-747 on tail-transection bleeding time were compared with clopidogrel and ticlopidine in SD rats. The bleeding time was measured 4 hr after the final dose of a 3-day repeated administration period of CS-747 (0.1–1 mg/kg/day,

p.o.), clopidogrel (1–10 mg/kg/day, *p.o.*), and ticlopidine (30–300 mg/kg/day, *p.o.*). All agents tested prolonged bleeding time in a dose-dependent manner (Fig. 7). Although CS-747 was the most potent in the prolongation of bleeding time, the relative potency among these agents was similar to that of *ex vivo* antiaggregating effects in SD rats. From these studies, CS-747 and clopidogrel may comparatively have similar ratios of benefit/bleeding risk. This might be important, since clopidogrel is clinically available and its benefit/bleeding risk ratio has been determined in a large clinical study.¹⁰ These results suggest that CS-747 is a potent antiplatelet agent with relatively moderate antihemostatic potency, but this remains to be proven in future clinical studies.

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4. Mode of Antiplatelet Action of CS-747

1) Reversibility of the effects of CS-747

The reversibility of CS-747-mediated inhibition on *ex vivo* platelet aggregation was examined in SD rats. The inhibition of platelet aggregation by CS-747 (3 mg/kg, *p.o.*) continued even after washing the platelets three times. In contrast, the inhibition of platelet aggregation by PGE₁ (1 μ M) or cilostazol (300 μ M) was reversed by washing the platelets three times. These data suggest that the antiplatelet action of CS-747 is irreversible. Moreover, the long duration of action of CS-747, even after a single oral administration, also supports this contention (Fig. 2). Hence, it is likely that CS-747 inhibits platelet aggregation in an irreversible manner.

2) Agonist selectivity

Agonist selectivity of the antiaggregatory activity of CS-747 was examined in *ex vivo* experiments in SD rats. Platelet aggregation was measured in the washed platelets from the SD rats treated with the vehicle or CS-747 (3 mg/kg, *p.o.*). ADP, collagen, and thrombin were used as agonists. CS-747 inhibited ADP- and collagen-induced platelet aggregation. CS-747 also caused a partial inhibition of thrombin-induced platelet aggregation, but not when a high concentration (0.3 unit/ml) of thrombin was used as the agonist. This inhibitory profile of CS-747 in rats was similar to apyrase, an ADP scavenger (data not shown).

In addition, several lines of evidence have suggested that collagen-induced rat

platelet aggregation is associated with ADP released from activated platelets. Collagen-induced platelet aggregation was not seen in Fawn Hooded rats,²⁰⁾ which have a congenital deficiency of ADP in their platelet-dense granules;²¹⁾ creatine phosphate/creatine phosphokinase, which converts ADP to ATP, completely inhibited collagen-induced platelet aggregation in rats.²²⁾ Thus, these results indicate that CS-747 has a broad spectrum of *ex vivo* antiaggregatory activity, and that this activity is mainly involved in inhibiting ADP-induced responses.

3) *Ex vivo* effects of CS-747 against ADP receptors in platelets

ADP induces platelet activation, including a shape change from disc to sphere, aggregation, and secretion of granule contents.⁹⁾ These responses are considered to be mediated by three distinct receptors, P2X₁, P2Y₁, and P2T_{AC} receptors (Fig. 8),^{8,23)} and it has been reported that coactivation of two different G-protein-coupled receptors, P2T_{AC} and P2Y₁, where neither of which can cause platelet aggregation by itself, is essential for ADP-induced platelet aggregation.²⁴⁾ Since 2-MeS-ADP is a stable agonist for P2Y₁ and P2T_{AC},^{8,23)} radiolabeled 2-MeS-ADP binding to platelets has often been used to clarify the effects of agents against ADP receptors. A single oral administration of CS-747 (3 mg/kg, *p.o.*) to SD rats produced a significant but partial inhibition of [³H]-2-MeS-ADP binding to platelets in the *ex vivo* study (Fig. 9). Orally administered CS-747 (10 mg/kg, *p.o.*) also partially inhibited [³⁵S]GTP γ S binding to the platelet

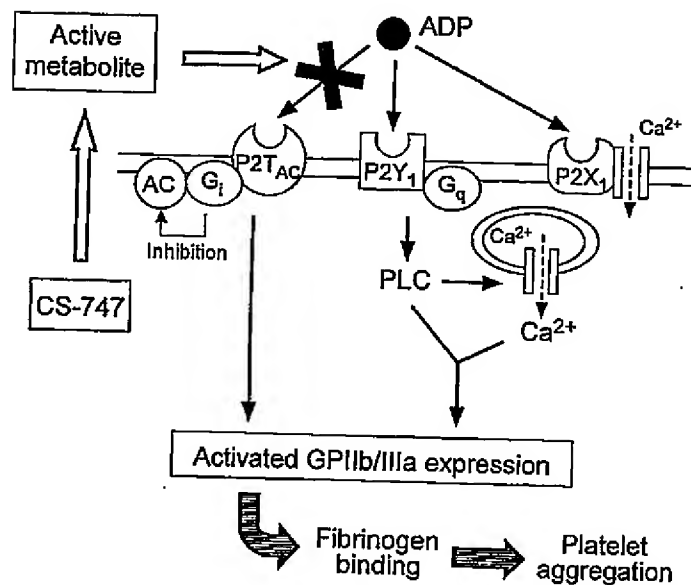


Fig. 8. ADP-induced signal transduction pathway and mechanism of anti-platelet action by CS-747 in platelets. AC: Adenyl cyclase; PLC: Phospholipase C.

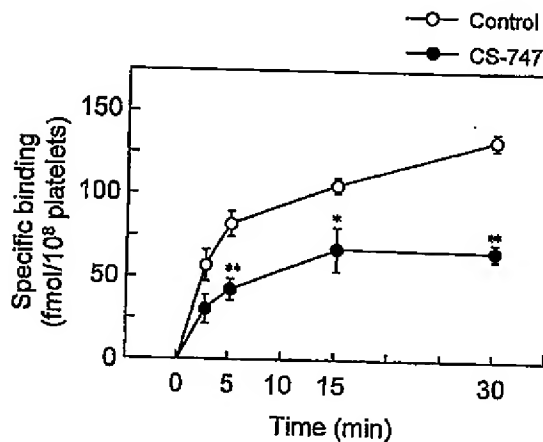


Fig. 9. Specific [³H]-2-MeS-ADP binding to platelets from vehicle-(open circles) and CS-747-treated rats (closed circles). CS-747 (3 mg/kg) was orally administered to rats 4 hr before blood collection. Results are expressed as the mean \pm S.E.M. (n=5). *p<0.05, **p<0.01 vs. control (vehicle-treated group).

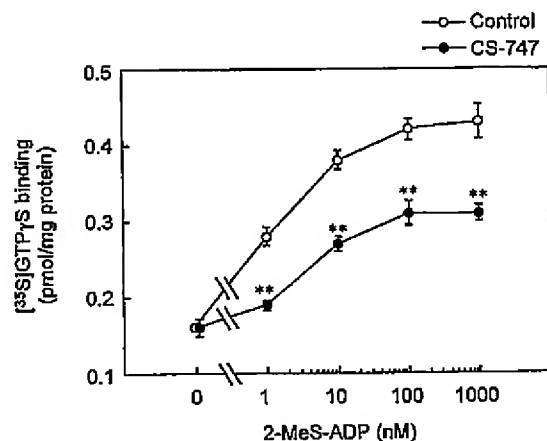


Fig. 10. [35 S]GTP γ S binding to platelet membranes from vehicle-(open circles) and CS-747-treated rats (closed circles). CS-747 (10 mg/kg) was orally administered to rats 4 hr before blood collection. [35 S]GTP γ S binding was measured 60 min after addition of radioligand to the membrane in the presence or absence of 2-MeS-ADP. Results are expressed as the mean \pm S.E.M. (n=5-6). **p<0.01 vs. control.

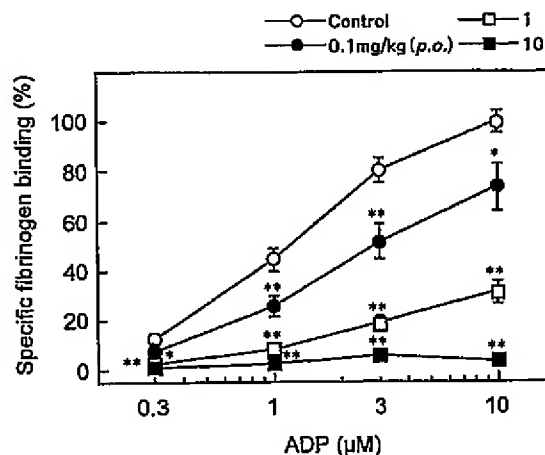


Fig. 11. *Ex vivo* effects of CS-747 (0.1-10 mg/kg, *p.o.*) on specific [125 I]-fibrinogen binding to ADP-stimulated rat platelets. CS-747 or vehicle was orally administered to rats 4 hr before blood collection. Fibrinogen binding was expressed as the percent of binding in 10 μ M ADP-stimulated platelets from control rats. Results are represented as the mean \pm S.E.M. (n=6). *p<0.05, **p<0.01 vs. control.

membrane (Fig. 10), indicating that an *ex vivo* inhibition of G-protein-coupled $P2T_{AC}$ or $P2Y_1$ receptors by CS-747 occurred.

In addition, our study confirmed that CS-747 neutralized the ADP-mediated inhibition of PGE_1 -induced adenylyl cyclase activation in platelets, which is mediated by $P2T_{AC}$ receptors, and CS-747 did not affect the $P2Y_1$ -mediated platelet shape change (data not shown). These results suggest that CS-747 inhibits platelet aggregation through selective interference of the $P2T_{AC}$ receptors on the platelet membrane.

4) *Ex vivo* effects of CS-747 on fibrinogen binding

Fibrinogen binding to activated glycoprotein IIb/IIIa (GPIIb/IIIa) in platelets is known as the final common step for platelet aggregation.²⁹ *Ex vivo* effects of

CS-747 on ADP-induced fibrinogen binding in SD rat platelets were examined. ADP (0.3–10 μ M) caused concentration-dependent [125 I]-fibrinogen binding to platelets in SD rats, which were given the vehicle. The [125 I]-fibrinogen binding induced by ADP was inhibited in platelets from CS-747 (0.1–10 mg/kg, *p.o.*)-treated SD rats (Fig. 11). This inhibition by CS-747 was dose-dependent. These data indicate that CS-747 inhibits platelet aggregation by preventing ADP-induced binding of fibrinogen to rat platelets through a $P2T_{AC}$ receptor blockade.

5) *Ex vivo* effects of CS-747 on Ca^{2+} mobilization

ADP-induced Ca^{2+} mobilization in platelets is induced by the activation of $P2Y_1$ and $P2X_1$ receptors.^{8,23} In the present study, *ex vivo* effects of CS-747 on ADP-

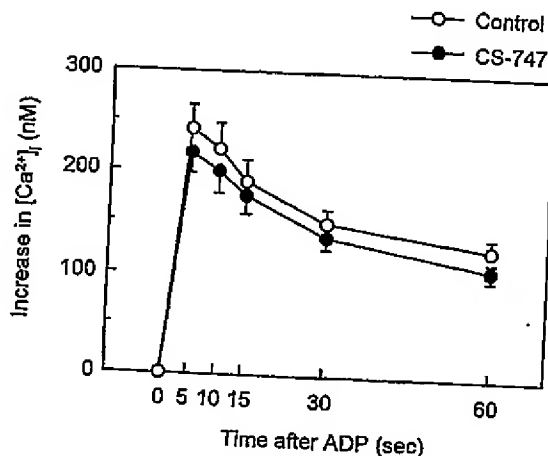


Fig. 12. *Ex vivo* effects of CS-747 (10 mg/kg, *p.o.*) on Ca^{2+} mobilization induced by 3 μ M ADP in platelets from vehicle-(control) and CS-747-treated rats in the presence of external 1 mM Ca^{2+} . Results are represented as the mean \pm S.E.M. ($n=13-14$). There were no significant differences between the two groups at any points.

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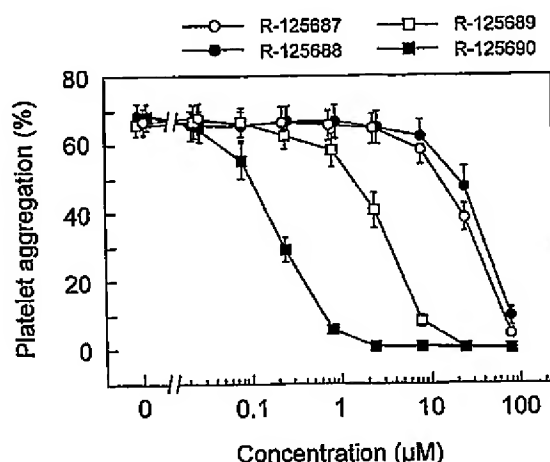


Fig. 13. *In vitro* effects of R-125687, R-125688, R-125689, and R-125690 on ADP (10 μ M)-induced platelet aggregation in washed human platelet suspensions. Results are represented as the mean \pm S.E.M. (n=6).

induced Ca²⁺ mobilization in SD rat platelets were examined 4 hr after dosing. In the presence of external Ca²⁺ (1 mM), ADP (3 μ M) induced an increase in intraplatelet Ca²⁺ concentrations. There were, however, no changes in ADP (3 μ M)-induced Ca²⁺ mobilization in the vehicle- nor CS-747 (10 mg/kg, *p.o.*)-treated SD rat platelets (Fig. 12). These data indicate that CS-747 has no activity on ADP-induced Ca²⁺ mobilization in platelets, which suggests that it possesses no P2Y₁ or P2X₁ antagonistic activity.

6) Active metabolite of CS-747

CS-747 (100 μ M) did not affect ADP-induced *in vitro* platelet aggregation of SD rat or human PRP but showed potent antiplatelet and antithrombotic effects *in vivo* as described above, suggesting the presence of an active metabolite for CS-

747. *In vitro* effects of R-99224, an *in vivo* metabolite of CS-747, on platelet aggregation were examined using PRP from several experimental animal species. R-99224 (0.753–75.3 μ M) inhibited *in vitro* platelet aggregation, in a concentration-dependent manner. The IC₅₀ values in SD rats, humans, cynomolgus monkeys, beagle dogs, and Japanese white rabbits were 44.9 μ M (rat), 15.2 μ M (human), 31.8 μ M (monkey), 7.3 μ M (dog), and 22.1 μ M (rabbit), which suggests that R-99224 is an active metabolite of CS-747. R-99224 also inhibited [³H]-2-MeS-ADP binding to platelets, and neutralized ADP-mediated inhibition of PGE₁-induced adenylyl cyclase activation in platelets (data not shown), suggesting that R-99224 has an antagonistic activity on P2T_{AC} receptors.

R-99224 is a mixture of two enantiomers: R-125688 (7a=S, 1'=R) and R-

125690 (7a=R, 1'=S). R-100364 (a mixture of two enantiomers: R-125687 (7a=S, 1'=S) and R-125689 (7a=R, 1'=R)) is the diastereomer of R-99224. *In vitro* activities of these four enantiomers on ADP-induced human platelet aggregation were examined using washed platelets. All of the enantiomers (0.0238–79.2 μ M) inhibited platelet aggregation in a concentration-dependent manner, with the following order of potency: R-125690 > R-125689 > R-125687 = R-125688 (Fig. 13). The IC_{50} values were 0.19 μ M for R-125690, 3.1 μ M for R-125689, 28 μ M for R-125687, and 36 μ M for R-125688. These results show that R-125690 has the most potent antiplatelet activity among the four enantiomers.

5. Conclusion

The results of the pharmacological studies indicate that CS-747 is an orally active antiplatelet agent, which produces dose-dependent and cumulative antiplatelet and antithrombotic effects with rapid onset and long duration of action. The effects of CS-747 are more potent than those of clopidogrel and ticlopidine. These potent activities are mainly mediated by an active metabolite: R-125690 (an enantiomer of R-99224), via a selective $P2T_{AC}$ receptor blockade. In addition, CS-747 has a relatively moderate antihemostatic potency at therapeutic doses. These data strongly suggest that CS-747 would be a useful antithrombotic agent in vascular thromboembolic disorders.

(Atsuhiko Sugidachi)

IV. Absorption, Distribution, Metabolism, and Excretion in Animals

The metabolism and pharmacokinetics of CS-747 were investigated after oral and intravenous administrations of labeled or non-labeled CS-747 to male Wistar-Imamichi rats, male beagle dogs, and male cynomolgus monkeys.

1. Absorption

1) Plasma concentration

^{14}C -CS-747 (Fig. 1) in solution (5% dimethylacetamide-95% polyethyleneglycol 400) was administered orally or intravenously to male rats at a dose of 5 mg/kg, and the plasma and blood concentrations of the radioactivity were determined using a liquid scintillation counter. The AUC (0–24 hr) value for the total radioactivity in plasma after oral administration was 54.73% of that after intravenous administration.

Assay methods by liquid chromatography/atmospheric pressure chemical ionization-mass spectrometry/mass spectrometry (LC/APCI-MS/MS) were established for the main plasma metabolites: the 2-oxo compound (R-95913), a metabolite produced before formation of the pharmacologically active SH compounds (R-104434 and R-99224), and the S-methyl compounds (R-106583 and R-100932), metabolites produced after the SH compounds, in the metabolic pathway (Chart 1). The quantitative measurement of the SH compounds was difficult to achieve due to their apparent instability, although

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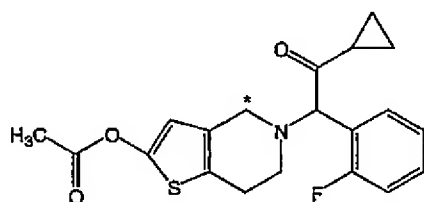


Fig. 1. Chemical structure of ^{14}C -CS-747
*Position labeled with ^{14}C

they were detectable in the plasma at a level of $\mu\text{g/ml}$. As shown in Fig. 2, all three metabolites described above were detected in plasma samples of rats, dogs, and monkeys after oral administration at a dose of 1 mg/kg. The linearity of the pharmacokinetics was demonstrated in dogs

after oral administration at doses of 0.5, 1.0, and 2.0 mg/kg (Fig. 3).

2) Absorption ratio

As will be described later, the ratio of absorption in rats, calculated as the ratio of the biliary excretion of the total radioactivity after oral administration of ^{14}C -CS-747 to that after intravenous administration, had a relatively high value of 67.6%. The absorption ratio in rats, evaluated by the urinary excretion ratios after oral and intravenous administration, was 85.2%. These results indicate high oral absorption after administration of CS-747.

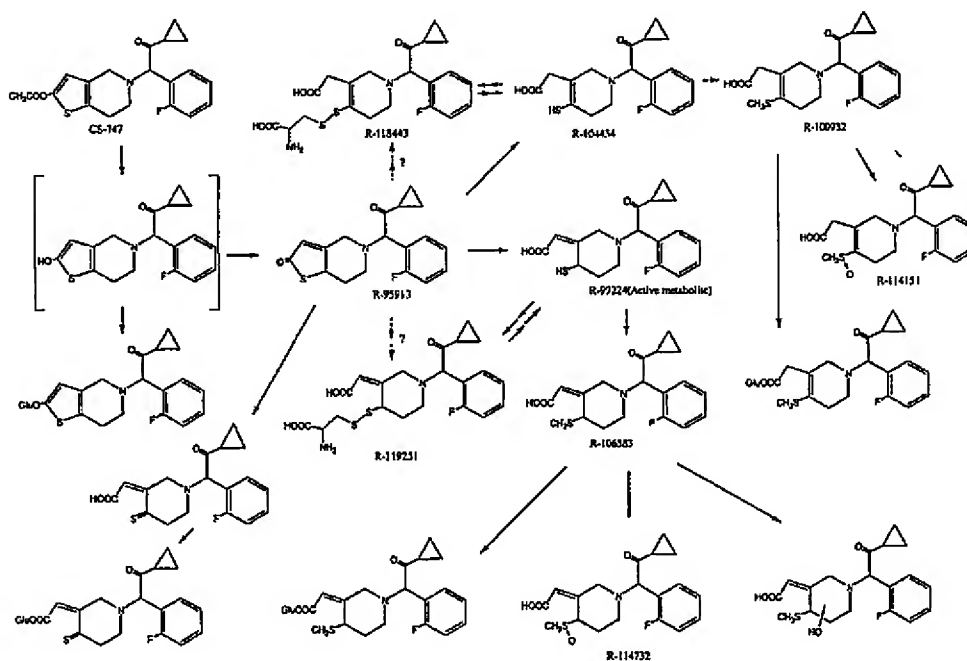


Chart 1. Proposed metabolic pathway of CS-747

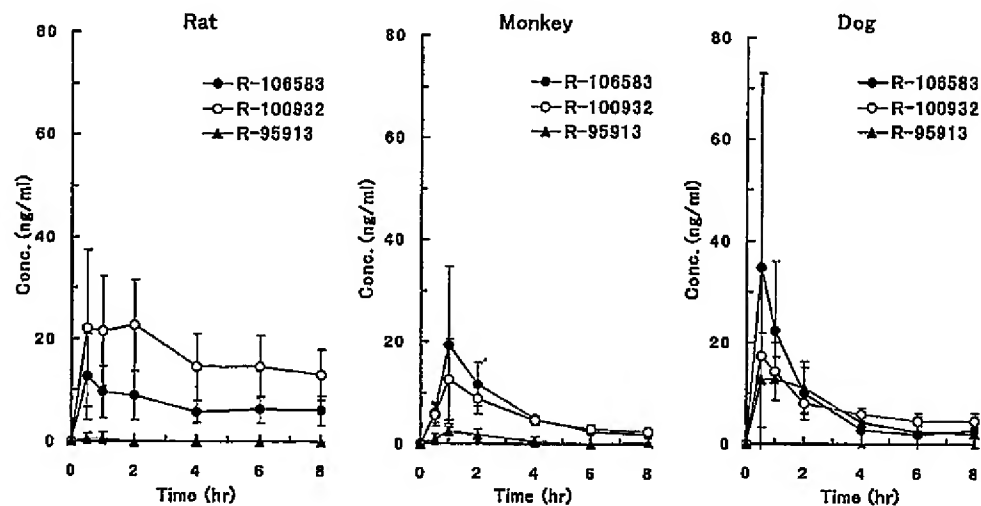


Fig. 2. Time courses of plasma concentrations of R-106583, R-100932 and R-95913 after a single oral administration of CS-747 to male rats, monkeys and dogs at a dose of 1 mg/kg. Data are represented as the mean \pm S.D.

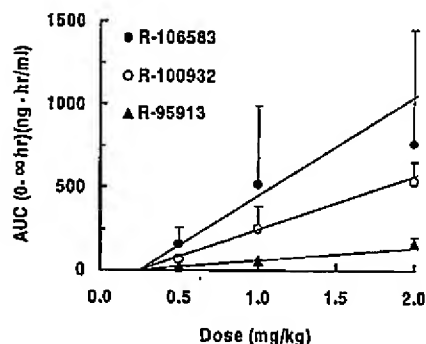


Fig. 3. Dose-AUC relationship after oral administration of CS-747 to dogs. Data are represented as the mean \pm S.D.

2. Distribution

After oral and intravenous administrations of ^{14}C -CS-747 in solution (5% dimethylacetamide-95% polyethyleneglycol 400) to male rats at a dose of 5 mg/kg,

one animal was sacrificed at each data point, and sagittal whole-body sections were prepared to investigate the distribution of the radioactivity by whole-body autoradiography.

At 30 min after oral administration (Fig. 4), the highest radioactivity was observed in the liver, followed by the kidneys, lungs, blood, adrenal glands, and heart. The concentrations in other tissues and organs were lower than the blood concentration. The concentration in the central nervous system was negligible. At 1 hr after oral administration, the liver still showed the highest radioactivity. The distribution profile of the radioactivity was very similar to that observed at 30 min after administration. At 3 hr after administration, the radioactivity in the animal body markedly decreased. The liver

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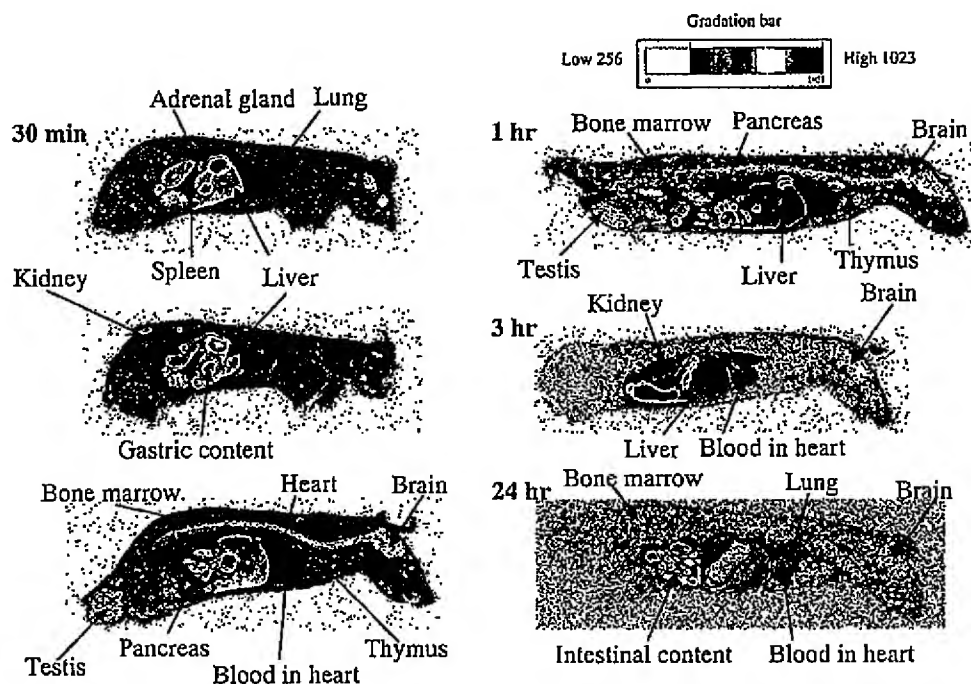


Fig. 4. Whole-body autoradiograms after oral administration of ^{14}C -CS-747 to rats at a dose of 5 mg/kg

showed the highest radioactivity, followed by the kidneys, lungs, adrenal glands, blood and heart. At 24 hr after administration, most of the radioactivity had been eliminated from the animal body. The highest radioactivity was observed in the intestinal contents, and relatively high radioactivity was observed in the liver, indicating enterohepatic circulation. Low radioactivity was also detectable in the blood and lungs.

At 30 min after intravenous administration (Fig. 5), the highest radioactivity was observed in the contents of the stomach, indicating gastric secretion. The distribution profile of the radioactivity was similar to that after oral administration. The liver

showed the highest radioactivity, followed by the kidneys, brown fat, lungs, adrenal glands, heart, and blood. Thereafter, distribution of the radioactivity in the whole body, over time, showed a similar course to that seen after oral administration.

3. Metabolism

The predicted metabolic pathway of CS-747, based on the biliary metabolites of rats and on the plasma metabolites of rats and dogs, is shown in Chart 1. The structures enclosed in the brackets are those for the intermediate metabolites which have not yet been isolated. The 2-oxo compound (R-95913), the SH compounds (R-104434 and R-99224), the S-

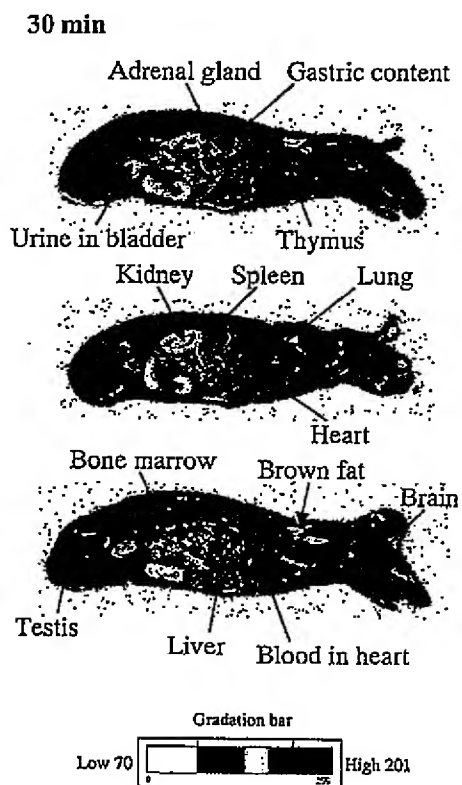


Fig. 5. Whole-body autoradiograms after intravenous administration of ^{14}C -CS-747 to rats at a dose of 5 mg/kg

methyl compounds (R-100932 and R-106583), and the disulfides of the SH compounds with cysteine (R-119251 and R-118443) were detected as the main plasma metabolites in rats and dogs. These metabolites were also detected in monkey plasma, as shown in Fig. 2, indicating that there is no marked species difference in the metabolic pathway. Of these plasma metabolites, only the SH compound (R-99224) showed anti-platelet activity *in vitro*, as described in the previous chapter

on pharmacology. The metabolites detected in the plasma of rats were also detected in their bile. The *S*-oxide form metabolites (R-114151 and R-114732) were found as predicted by the metabolism reported for other sulfur-containing xenobiotics. Hydroxylated metabolites of the *S*-methyl compounds at an unknown position of the piperidine ring were also detected.

Therefore, the major metabolic pathway of CS-747 was considered to consist of the following reactions: deacetylation (hydrolysis) of CS-747 to the 2-oxo compound (R-95913); thiolactone ring-opening reaction of the 2-oxo compound to the SH compounds (R-104434 and R-99224); disulfide formation of the SH compounds with cysteine (R-119251 and R-118443); *S*-methylation of the SH compounds to the *S*-methyl compounds (R-100932 and R-106583); *S*-oxidation of the *S*-methyl compounds to the *S*-oxide compounds (R-114151 and R-114732); and hydroxylation or glucuronidation of the *S*-oxide compounds.

Accumulating evidence indicates that the conversion of the 2-oxo compound to the SH compounds, which is the metabolic process producing the pharmacologically active metabolite, is catalyzed by CYP3A4, the isoform of cytochrome P450 present in almost all humans. The disulfide-type cysteine conjugates of the SH compounds were also the main metabolites in the bile, and indicated the presence of disulfide-type glutathione conjugates of the SH compounds as intermediate metabolites. The cysteine conjugate of R-99224 (R-119251) exhibited anti-platelet activity *in vivo*, which was considered to

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be due to the SH compound (R-99224) produced by a reduction in the presence of an excess amount of reduced glutathione in cells. The glucuronic acid conjugate at the 2-hydroxyl group was isolated from rat bile, indicating transient occurrence of the 2-hydroxy compound immediately after hydrolysis of CS-747 (the intermediate metabolite in brackets). The 2-thiocarbonyl compound was detected predominantly *in vitro*. However, the glucuronide of the 2-thiocarbonyl compound was detected in the bile, demonstrating that this metabolism also occurs *in vivo*.

Ticlopidine and clopidogrel, the prototype drugs in this therapeutic field, do not exhibit anti-platelet activity as such, and have been indicated to produce active metabolites through the action of cytochrome P450. Despite many efforts, however, the active metabolites of ticlopidine and clopidogrel have not been successfully isolated. Based on the CS-747 metabolism, it is quite likely that the corresponding SH-form metabolites are produced from these two drugs as their pharmacologically active metabolites *in vivo*. In our preliminary *in vitro* experiments using rat liver, the mass spectrometric peak of the clopidogrel metabolite that possesses exactly the same molecular weight as that calculated for the predicted SH-form metabolite for clopidogrel has been found. Production of this assumed active metabolite from clopidogrel was low, at a level of about 1/10 of the production of the SH compound from CS-747 under the same conditions. The assumed active metabolite of clopidogrel is thought to require 2 successive enzymatic oxida-

tions of the parent drug, namely, the formation of the 2-oxo form from clopidogrel followed by conversion of the 2-oxo form to the SH form. In contrast, the formation of R-99224, the SH compound of CS-747, needs only a single oxidation of R-95913, the 2-oxo compound, and therefore, a rapid and extensive production of the active metabolite from CS-747 compared to clopidogrel is quite convincing.

4. Excretion

Up to a period of 72 hr after oral administration of ¹⁴C-CS-747 in solution to male Wistar-Imamichi rats (5 mg/kg), 20.7 ± 0.9% and 73.2 ± 7.3% (mean ± S.D.) of the dose administered were excreted in urine and feces, respectively (total recovery ratio: 93.9 ± 8.1%). After intravenous administration, 24.3 ± 0.6% and 74.5 ± 1.7% of the dose were excreted in urine and feces, respectively (total recovery ratio: 98.8 ± 1.0%).

Up to a period of 24 hr after oral and intravenous administration of ¹⁴C-CS-747 to bile-fistula rats (5 mg/kg), the amounts of radioactivity excreted in the bile were 51.29 ± 1.65% and 75.89 ± 3.82% of the dose, respectively.

Up to a period of 144 hr after oral administration of ¹⁴C-CS-747 in solution to male beagle dogs (5 mg/kg), 31.2 ± 0.3% and 60.3 ± 1.2% of the dose administered were excreted in urine and feces, respectively (total recovery ratio: 91.4 ± 1.5%).

Therefore, the main route of excretion was demonstrated to be the fecal pathway *via* biliary excretion, both in rats and dogs.

5. Conclusion

CS-747 was metabolized extensively in rats, dogs, and monkeys, and the unchanged compound was not detected in plasma in any of the three animal species. The 2-oxo compound, SH compounds, and S-methyl compounds were detected in common as the main metabolites in plasma in these animal species. Relatively good absorption of CS-747 in rats was indicated, as judged by biliary and urinary excretions of the total radioactivity after oral administration in comparison to those after intravenous administration (68.93 and 85.19%, respectively). The S-oxide compounds, as well as the hydroxylated and/or conjugated metabolites, were detected in rat bile, demonstrating that metabolism proceeds further. Of the metabolites found in the plasma and bile, only the SH compounds exhibited the anti-platelet activity *in vitro*. Distribution of the radioactivity was high in the liver, kidneys, adrenal glands, blood, lungs and heart, while almost no uptake by the central nervous system was observed after administration to rats. About 60–75% of the dose and about 25–30% of the dose were excreted in the feces and urine, respectively, after administration to rats and dogs.

(Toshihiko Ikeda)

V. Safety Evaluations in Animals

1. Single-dose and Escalating-dose Studies

The methods and results obtained are summarized in Table 1.

1) Single-dose study in mice and rats

CS-747 was orally administered to RFVL mice at a dose of 2000 mg/kg. No animals died during the study period. No changes in clinical signs were observed in any animals. Excretion of yellowish-brown urine within 2 days after administration was observed in all animals. There were no adverse effects observed in gross pathological examinations of any animals at autopsy.

CS-747 was orally administered to F344 rats at doses of 1000 or 2000 mg/kg. No animals died during the study period. Mydriasis and yellow-brown changes in urine color were observed in all animals. Irregular respiration, reduction of locomotor activity, ptosis, lacrimation, and staggering gait were observed in female rats that received 2000 mg/kg. There were no adverse effects observed in gross pathological examinations of any animals at autopsy.

From the above results, CS-747 is considered to be relatively non-toxic in mice and rats after acute administration of doses up to 2000 mg/kg.

2) Increasing-dose study in dogs

CS-747 was administered orally at daily increasing doses of 10, 30, 100, 300, 1000 and 2000 mg/kg to one animal of each sex in beagle dogs. Animals were observed for 14 days after administration of 2000 mg/kg. Emesis was observed after administration at doses of 300 mg/kg or more. Inhibition of platelet aggregation, which was thought to be attributable to pharmacological action, was observed in hemato-

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Table 1. Summary of single-dose toxicity studies

Species (Strain) Sex/Group Total Age	Route (Vehicle) [Dose volume]	Dose (mg/kg)	Minimum lethal dose (mg/kg)	Results
Mouse (RFVL) 10M+10F 20 4 weeks	Oral (0.5% TG) [20 ml/kg]	2000	>2000	-Yellowish brown urine (all animals)
Rat (F344) 5M+5F 20 5 weeks	Oral (0.5% TG) [10-20ml/kg]	1000 2000	>2000	-Mydriasis, yellowish brown urine (all animals) -Irregular respiration, reduction of locomotor activity, lacrimation, and staggering gait (females, 2000 mg/kg)
Dog* (Beagle) 1M+1F 2 16 and 11 months	Oral (GC)	10 30 100 300 1000 2000	>2000	-Emesis (>300mg/kg) -ALP ↑, atrophy of liver cells, ground-glass appearance of liver cells (2000 mg/kg)

M=male; F=female; TG=Tragacanth gum; GC=gelatin capsule; ALP=alkaline phosphatase.

*Increasing-dose study.

logical examination. After administration of 2000 mg/kg, serum biochemical analysis revealed an increase in alkaline phosphatase; furthermore, atrophy of liver cells and a ground-glass appearance of liver cell cytoplasm were evident in pathological examinations.

2. Repeated-dose Studies

The methods and results are summarized in Table 2.

1) Three-month oral administration in rats

CS-747 was orally administrated to

groups of ten F344 rats of each sex at dosage levels of 0, 10, 30, 100, or 300 mg/kg/day for 3 successive months. Toxicological evaluation was carried out by analyzing the results of the following examinations: general conditions, body weight, food intake, hematology, blood chemistry, ophthalmology, and pathology.

No animals died during the study period. The following changes were attributed to the effects of CS-747 treatment, based on differences compared with the control level and dose dependency: the color of urine (yellow), in the 300 mg/kg male and female groups; decreases in body weight,

Table 2. Summary of repeated-dose toxicity studies (rat and dog)

Species (Strain) Sex/Group Total Age	Route (Vehicle) [Dose vol.]	Daily dose (mg/kg)	Duration	Highest non-toxic dose (mg/kg)	Results
Rat (F344/Du Crj) 10M+10F 100 7 weeks	Oral (0.5% TG) [5 ml/kg]	VC 10 30 100 300	3 months	100	-Yellow urine (300 mg/kg) -Body weight ↓ (300mg/kg) -Platelet number ↑ (males, >100mg/kg; females, 300 mg/kg) -Elongation of prothrombin time (males, >30 mg/kg) -Elongation of activated partial thromboplastin time (>100 mg/kg) Liver weight ↑ (males, >100 mg/kg; females, >30 mg/kg) -Hypertrophy of liver cells (males, >100 mg/kg; females, 300 mg/kg)
Dog (Beagle) 3M+3F 24 10-20 months	Oral (GC)	VC 0.8 4 20	3 months	4	-Hypertrophy and ground-glass appearance of liver cells (>4 mg/kg) -Plasma ALP ↑, total cholesterol ↓ (20 mg/kg) -Slight proliferation of smooth endoplasmic reticulum (20 mg/kg)

M=male; F=female; VC=vehicle control; TG=Tragacanth gum; GC=gelatin capsule.

in the 300 mg/kg male and female groups; increases in the number of platelets, in the 100 mg/kg or more male and 300 mg/kg female groups; prolongation of prothrombin times, in the 30 mg/kg or more male groups; prolongation of activated partial thromboplastin times, in the 100 mg/kg or more male and female groups; increases in liver weight, in the 100 mg/kg or more male and 30 mg/kg or more female groups; and hypertrophy of liver cells, in the 100 mg/kg or greater male and 300 mg/kg female groups.

Therefore, the non-toxic dose of CS-

747 in the present study was determined to be 100 mg/kg.

2) Three-month oral administration in dogs

CS-747 was administered, in gelatin capsules, at doses of 0, 0.8, 4, or 20 mg/kg, for 3 months to groups of beagle dogs (three male and three female dogs per group). Clinical signs, body weight, food intake, water intake, and urine volume were recorded, and liver function tests, kidney function tests, electrocardiography, ophthalmological examination,

electroretinography, urinalysis, hematological and blood biochemical examinations, and pathological examinations, including organ weight, gross pathology, and histopathology, were performed.

In animals that received 0.8 mg/kg or more, decreased platelet aggregation activity, as the main pharmacological effect, was noted. In animals that received 4 mg/kg or more, hypertrophy of hepatocytes, accompanied by a ground-glass appearance of cytoplasm, was observed in the liver. Animals that received 20 mg/kg showed increased alkaline phosphatase activities and decreased total cholesterol levels in the blood biochemical examination. Electron microscopic examination, conducted on the dosing group, revealed a slight proliferation of smooth surface endoplasmic reticulum in hepatocytes.

It was concluded that 4 mg/kg and 20 mg/kg represent non-toxic and toxic dose levels, respectively, in the present study.

3. Mutagenicity

1) Gene mutation assay of CS-747 in bacteria

Tests were carried out with four *Salmonella typhimurium* strains, TA1535, TA1537, TA98, and TA100, and an *Escherichia coli* strain, B/r WP2 (WP2uvrA), in combination with or absence of an S9 mixture, which is comprised of a cofactor mixture with a post-mitochondrial fraction (S9) derived from male rat liver. The reverse mutation test was conducted at doses of 200, 500, 1000, 2000, and 5000 µg/plate, with 4 plates for each dose. There was no increasing response in the numbers of revertants in

the test strains, either with or without the metabolic system. Therefore, CS-747 was estimated not to have mutagenic activity detectable in the test system adopted herein.

2) Chromosome aberration test of CS-747

A chromosome aberration study on CS-747 was performed in a cell line established from the lung of a female Chinese hamster (CHL), to investigate possible genotoxicity. As a result, there were no significant changes in the incidence of cells possessing abnormal chromosomes, either in the direct method or the metabolic activation method. CS-747 was considered not to induce chromosome aberrations under these test conditions.

3) Micronucleus test of CS-747 in mice

The micronucleus test on CS-747 in male ICR mice was performed to investigate possible genotoxicity. The micronucleus test was performed at doses of 400, 800, or 1600 mg/kg, based on results obtained from a dose-finding study. Since four out of six animals died at 1600 mg/kg, a 1000-mg/kg group was additionally incorporated. All treatment groups showed no statistically significant increase in micronucleated polychromatic erythrocyte counts. CS-747 was shown to be devoid of micronuclei-inducing activity.

4. Antigenicity

1) Antigenicity study in guinea Pigs

The CS-747 single oral-administration groups (0.3 mg/body and 3 mg/body) and the CS-747 plus Freund's complete adju-

vant (FCA) subcutaneous-administration groups (0.3 mg/body and 3 mg/body) of guinea pigs were provided as the immunized groups, and sodium 2, 4, 6-trinitrobenzenesulfonate dihydrate (TNBS) plus FCA subcutaneous-administration group (3 mg/body) was employed as the positive control. Antibody titer by passive cutaneous anaphylaxis (PCA) was not observed in any animals of the CS-747 single oral- or CS-747 plus FCA subcutaneous-administration groups. Systemic anaphylaxis (SANA) was also negative in all the immunized groups.

Therefore, CS-747 is not considered to exhibit antigenicity.

2) Antigenicity study in mice

The CS-747 single oral-administration groups (0.1 mg/body and 1 mg/body) and the CS-747 plus alum intraperitoneal-administration groups (0.1 mg/body and 1 mg/body) of A/J mice were provided as the immunized groups, and a TNBS plus alum intraperitoneal-administration group (0.3 mg/body) was employed as the positive control. IgE titer by rat PCA reaction was not observed in any animals of the CS-747 single oral- or CS-747 plus alum intraperitoneal-administration groups.

Therefore, CS-747 is not considered to exhibit antigenicity.

5. Reproductive Toxicity Studies

1) Fertility study in rats

CS-747 was orally administered, at doses of 30, 100, or 300 mg/kg, to groups of 24 male and 24 female Crl:CD rats. Males were treated for 4 weeks prior to and throughout mating to termination.

Females were treated for 2 weeks prior to and throughout mating up to and including Day 7 of presumed pregnancy. On Day 15 of presumed pregnancy, females were sacrificed, subjected to postmortem examination, and litter values were determined. Following the sacrifice of the females on Day 15 of presumed pregnancy, males were sacrificed and subjected to postmortem examination.

Treatment was associated with bright yellow staining of the tray paper under all cages of females in the groups given 30 mg/kg or more. In the 100 mg/kg group, body weight gains of both sexes during the pre-mating treatment period were significantly lower than in the controls. There were no obvious adverse effects on fertility or early embryonic development to implantation in any groups.

Based on the above results, the non-toxic dose, in terms of general toxicity in parent animals, is considered to be 30 mg/kg, and that in terms of maternal reproductivity and development of the next generation, is considered to be more than 300 mg/kg.

2) Teratogenicity study in rats

CS-747 was orally administered, at doses of 30, 100, or 300 mg/kg, to groups of 24 mated female Crl:CD rats, once daily, from Days 7 to 17 inclusive of presumed pregnancy. On Day 20 of presumed pregnancy, animals were sacrificed and subjected to postmortem examination, litter values were determined, and fetuses were examined for gross external abnormalities. Fetuses were subsequently sexed and subjected to detailed visceral or skele-

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A significant reduction in food intake during the first 7 days of treatment, and higher water intake during the first 3 days of treatment, were also observed in the 300 mg/kg group. There was a slight but significant reduction in mean fetal weight in the 300 mg/kg group. There were no obvious adverse effects on the development of the fetuses in the 30 or 100 mg/kg groups.

Based on the above results, the non-toxic dose, in terms of general toxicity in pregnant females, is considered to be 30 mg/kg, and that in the terms of maternal reproductivity is considered to be more than 300 mg/kg. The non-toxic dose for development of the next generation is considered to be 100 mg/kg.

6. Comparative Toxicity Studies

The methods and results are summarized in Table 3.

1) Toxicity study on CS-747 and clopidogrel in rats

A study was conducted to compare the oral toxicity of CS-747 and clopidogrel. CS-747 or clopidogrel was given orally to groups of 10 male and 10 female F344 rats for 28 consecutive days. Dose levels were 100 or 400 mg/kg for each compound. The following changes were attributed to the effects of CS-747 or clopidogrel treatment, based on differences compared with the control: mydriasis, suppression of body weight gain, and decreases in food intake, in the groups given 400 mg/kg of CS-747; salivation and decreases in food intake, in the groups given 400

mg/kg of clopidogrel; and the color of urine (yellow), in all groups given either substance. In the hematological or blood biochemical examinations, the following changes were observed in all groups given either substance: lower red blood cell counts, hematocrit, hemoglobin, alkaline phosphatase, total bilirubin, and triglyceride; higher platelet counts, total cholesterol, total protein, albumin, and calcium; and elongation of activated partial thromboplastin times. Low values of glucose, and high values of urea nitrogen, were observed in the groups given CS-747. In the pathological examination, the following were observed: increases in liver weight, in the group given 100 mg/kg of clopidogrel, and in the groups given 400 mg/kg of either compound; hypertrophy of liver cells, and hypertrophy of follicular epithelium of the thyroid, in all groups given either compound; proliferation of smooth endoplasmic reticulum (SER) of liver cells, in the groups given 400 mg/kg of either compound; and regeneration of tubular epithelium, and erythrocyte casts and acidophilic crystals in the tubular lumens of the kidney, in the group given 400 mg/kg of clopidogrel. In summary, although several changes attributable to dosing of CS-747 or clopidogrel were observed, no differences in the quality or intensity of the changes were found between animals given either compound.

Therefore, it was concluded that there are no significant differences in the toxicity of CS-747 and clopidogrel to rats. However, it was concluded that CS-747 is less toxic than clopidogrel in terms of effect on the kidney.

Table 3. Summary of comparative toxicity studies on CS-747 and clopidogrel

Species (Strain) Sex/Group Age	Route (Vehicle) [Dose vol.]	Daily dose (mg/kg)	Duration	Results
Rat (F344/Du Crj) 10M+10F 20 7 weeks	Oral (0.5% TG) [5 ml/kg]	VC CS-747 or Clopidogrel 100 400	28 days	<u>At 400 mg/kg of CS-747</u> Suppression of body weight gain <u>At 400 mg/kg of clopidogrel</u> -Salivation, food consumption ↓ -Regeneration of fibula epithelium and erythrocyte casts and acidophilic crystals in the kidney <u>At 400 mg/kg of both</u> -Yellow urine, food consumption ↓ -Plasma RBC counts ↓, hematocrit ↓, hemoglobin ↓, ALP ↓, total bilirubin ↓, triglyceride ↓, platelet counts ↑, total cholesterol ↑, total protein ↑, albumin ↑ -Liver weight ↑ -Hypertrophy of liver cells and follicular epithelium of the thyroid -Proliferation of SER in liver
Dog (beagle) 2M+2F 7-8 months	Oral (GC)	VC CS-747 or Clopidogrel 4 20 50 100	28 days	<u>At 20 mg/kg or more of Clopidogrel</u> -Emesis -Erosion, ulcer formation, regeneration in mucous membrane of the stomach <u>At 20 mg/kg or more of CS-747</u> -Increase of ALP value in plasma <u>At all doses of both</u> -Ground-glass appearance in cytoplasm in liver -Increase of P-450 content and decrease of ACD in liver, liver weight ↑

M=male; F=female; TG=Tragacanth gum; GC=gelatin capsule; VC=vehicle control; ALP=alkaline phosphatase.

2) Toxicity study on CS-747 and clopidogrel in dogs

CS-747 or clopidogrel was administered to beagle dogs over a period of 28 days. The dose levels of CS-747 and clopidogrel were 4, 20, 50, or 100 mg/kg. Each group consisted of two subjects of

each sex. During the administration period, observation of clinical signs, measurement of body weight and food consumption, urinalysis, liver function tests, hematological examinations, and blood biochemical examinations were carried out. At the termination of the dosing period, pathological examination and measure-

ment of drug-metabolizing enzymes in the liver were conducted.

After administration of clopidogrel, vomiting was observed, and pathological examination revealed erosion, ulcer formation and regeneration in the mucous membrane of the stomach in animals that received 20 mg/kg or more. These were considered to be clopidogrel-specific toxic changes. There were no specific toxic changes after administration of CS-747. Treatment-related changes, which were seen after administration of both compounds, included: reduced platelet aggregation on hematological examination; increased alkaline phosphatase activity on blood biochemical examination; increased P450 content and decreased 7-alkoxycoumarin O-dealkylase (ACD) activity in the liver; increased liver weight, hypertrophy and ground-glass appearance in the cytoplasm of hepatocytes in the pathological examination; and proliferation of smooth endoplasmic reticulum (SER) and formation of membrane whirls in hepatocytes on electron microscopy. There were no considerable differences in the degree or frequency of appearance of these changes between the two compounds.

Based on these results, it was concluded that CS-747 has a less toxic effect than clopidogrel, since toxic changes were seen in the stomach after administration of clopidogrel.

7. Conclusion

The toxicology studies indicate that the highest non-lethal single dose of CS-747 is 2000 mg/kg in mice and rats; therefore, the minimum lethal dose in mice and rats

is more than 2000 mg/kg. Accordingly, the lowest toxic dose after a 90-day treatment in rats is 100 mg/kg, and that in dogs is 4 mg/kg. CS-747 has no mutagenicity, antigenicity, nor teratogenicity. The comparative studies conclude that CS-747 is less toxic than clopidogrel in rats and dogs.

(Atsushi Sanbuissho)

VI. Clinical Evaluation (Clinical pharmacology studies)

Three studies in Phase I: single-dose, multiple-dose, and food-effect studies of CS-747, were performed at Inveresk Clinical Research Ltd. in the U.K., to characterize the pharmacodynamics, pharmacokinetics, and safety features of CS-747, after single and multiple oral administrations in healthy Caucasian male volunteers. Table 1 shows protocol synopses for the studies.

1. Pharmacokinetics

1) Assay methodology

A validated LC-MS/MS was developed for quantification of the three major metabolites of CS-747: R-95913, R-100932, and R-106583, in human plasma. The metabolites, instead of the pharmacologically active, SH compound (R-99224, see the previous chapter on ADME), in plasma were analyzed following solid phase extraction. There was no component present in the human plasma that interfered with the quantification. The limit of quantification for the three metabolites was 1.56 ng/ml in human plasma.

Table 1. Protocol synopses of the CS-747 Phase I studies

Study	Single-dose	Food-effect	Multiple-dose
Design	Double-blind Placebo-controlled	2-way crossover (single dose)	Double-blind Placebo-controlled 10 days (once daily)
Subjects	Healthy males (n=5)	Healthy males (n=6)	
Dose	2.5, 10, 30, 75 mg Placebo	25mg	2.5, 10 mg Placebo
Evaluation			
1. PD ¹⁾	Platelet aggregation (ADP, collagen) Bleeding time (Ivy method)		
2. PK ²⁾	The main metabolites in plasma		
3. Safety	Hematology, clinical chemistry, urinalysis ECG, fundoscopy, examination for petechiae, checking vital signs, etc.		

¹⁾Pharmacodynamics; ²⁾Pharmacokinetics.

2) Pharmacokinetic profiles

i) Single-dose study

CS-747 was orally administered to healthy Caucasian male volunteers at single doses of 2.5, 10, 30, and 75 mg. Plasma concentrations of the three metabolites after administration in the fasting state at a dose of 10 mg are shown in Fig. 1, and the pharmacokinetic parameters are listed in Table 2. The maximum plasma concentration (C_{max}) and the area under the plasma concentration (AUC_{0-24}) of the metabolites increased proportionally to the dose administered, from 2.5 to 75 mg (Fig. 2). On the other hand, the other pharmacokinetic parameters, the time to reach maximum plasma concentrations (T_{max}), and the mean residence time (MRT), of the metabolites were almost similar to those of the 10-mg dose group. These three metabolites appeared in the plasma soon after administration, and reached the C_{max}

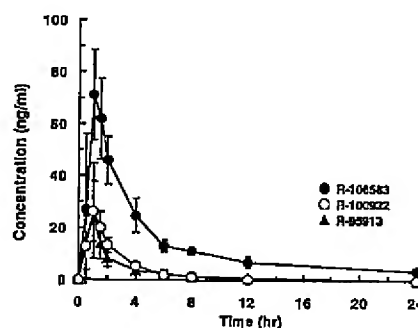


Fig. 1. Plasma concentrations of the metabolites after a single administration of CS-747 at a dose of 10 mg (mean \pm S.D.)

in 1 hr. The metabolites were rapidly eliminated from the plasma, with MRTs of 2–6 hr after the T_{max} . The most abundant metabolite was R-106583, which is an *S*-methylated compound of the active metabolite (R-99224, see the previous chapter on pharmacology). The concentration in human plasma was much higher than those in the laboratory animals tested.

Table 2. Pharmacokinetic parameters of the metabolites after a single administration of CS-747 at a dose of 10 mg

	R-95913	R-106583	R-100932
AUC ₀₋₂₄ (ng·hr/ml)	53.2 ± 23.8	324.5 ± 57.3	62.4 ± 21.7
C _{max} (ng/ml)	30.0 ± 15.4	71.5 ± 17.3	28.9 ± 8.8
T _{max} (hr)	0.8 ± 0.3	1.1 ± 0.2	0.9 ± 0.2
MRT ₀₋₂₄ (hr)	2.5 ± 1.0	6.3 ± 0.7	2.3 ± 0.7

Data are expressed as the mean ± S.D.

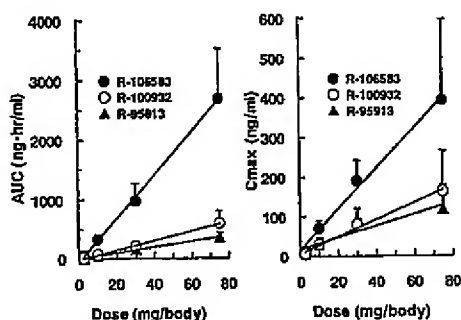


Fig. 2. Relationship between the dose and PK parameters of the three metabolites after single administrations of CS-747 (mean ± S.D.)

These results indicate that the gastrointestinal absorption of CS-747 is rapid and proportional to the dose tested, after a single administration. It was suggested that CS-747 was efficiently converted to the active metabolite soon after absorption, and that this metabolic pathway was more dominant than the other pathway, especially in humans, in comparison with the animals.

ii) Food-effect study

The effect of food on pharmacokinetics was evaluated in 6 healthy male volunteers after a single oral administration of CS-

747 at a dose of 25 mg, in a crossover study. The pharmacokinetic parameters of the most abundant metabolite, R-106583, are listed in Table 3. Food intake significantly delayed T_{max} by 1 to 2 hr. But there were no significant differences in the AUC₀₋₂₄, C_{max}, and MRT₀₋₂₄ for this metabolite between the fed and fasting regimens. This result indicates that meals hardly affect the pharmacokinetic profile of CS-747, except for the 1- to 2- hr delay in the absorption.

iii) Multiple-dose study

CS-747 was orally administered to healthy male volunteers, after breakfast, at doses of 2.5 and 10 mg, once daily for 10 days. A series of blood samplings on Days 1, 5, and 10, and also trough sampling before dosing on Days 2, 3, 6, and 8, were performed to investigate the steady-state plasma concentration of the metabolites. The plasma concentration profile of the metabolites during the treatment of CS-747 is shown in Fig. 3. Pharmacokinetic parameters of the metabolites are listed in Table 4. There was little difference in the pharmacokinetic profiles of CS-747 between Days 1, 5, and 10, suggesting that there was no accumulation of the metabo-

lites after multiple administration. The plasma concentration of the metabolites reached a steady-state by Day 3. The AUC_{0-24} and the C_{max} for the metabolites increased in proportion to the given doses of 2.5 and 10 mg of CS-747. The T_{max} and MRTs of the metabolites were similar between the different dose groups.

2. Pharmacodynamics

1) Assay methodology

Ex vivo platelet aggregation in platelet-rich plasma (PRP) induced by 20 μ M ADP or 2 μ g/ml of collagen was measured by the method of Born *et al.*²⁶⁾ using a Bio-

Data PAP-4 platelet aggregometer. The PRP after preincubation for about 3 minutes at 37°C was mixed with 10 μ l of agonist solution, and the resulting aggregation was monitored for about 4 min. Each sample was assayed in duplicate for each agonist.

Bleeding time was measured according to the Ivy method.²⁷⁾

2) Inhibition of platelet aggregation

i) Single-dose study

As shown in Fig. 4, a more than 50% inhibition of ADP-induced platelet aggregation was achieved 2 hr after single CS-

Table 3. Pharmacokinetic parameters of R-106583 in the food-effect study

	Fed	Fasted
AUC_{0-24} (ng·hr/ml)	916.8 \pm 113.7	862.8 \pm 151.2
C_{max} (ng/ml)	153.9 \pm 30.5	169.0 \pm 45.5
T_{max} (hr)	2.5 \pm 1.2	1.2 \pm 0.3
MRT ₀₋₂₄ (hr)	7.5 \pm 1.2	6.7 \pm 0.6

Data are expressed as the mean \pm S.D.

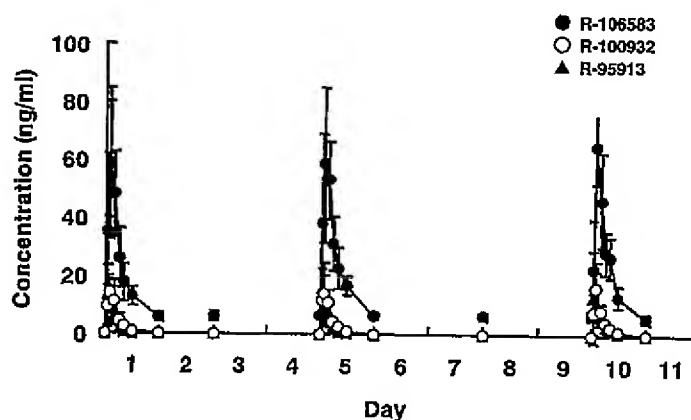


Fig. 3. Plasma concentrations of the metabolites after multiple administrations of CS-747 at a dose of 10 mg (mean \pm S.D.)

Table 4. Pharmacokinetic parameters of the metabolites in the multiple-dose study

10 mg dose				
Day 1		R-95913	R-106583	R-100932
T _{max}	(hr)	1.5 ± 0.6	2.3 ± 0.8	2.3 ± 0.8
C _{max}	(ng/ml)	17.1 ± 7.0	66.4 ± 18.3	16.7 ± 5.4
AUC _{0-∞}	(ng·hr/ml)	51.7 ± 11.1	549.6 ± 133.8	73.0 ± 21.9
Day 5				
T _{max}	(hr)	1.7 ± 1.2	2.5 ± 1.2	2.3 ± 1.4
C _{max}	(ng/ml)	21.9 ± 14.2	72.3 ± 11.5	17.6 ± 5.3
AUC _{0-∞}	(ng·hr/ml)	64.9 ± 26.9	537.2 ± 95.2*	79.0 ± 18.9
Day 10				
T _{max}	(hr)	1.7 ± 0.5	2.2 ± 1.0	1.8 ± 0.4
C _{max}	(ng/ml)	22.3 ± 12.1	68.6 ± 12.1	17.0 ± 6.1
AUC _{0-∞}	(ng·hr/ml)	69.4 ± 36.0	495.4 ± 89.1*	79.4 ± 20.9
2.5 mg dose				
Day 1				
T _{max}	(hr)	1.7 ± 0.5	2.2 ± 1.0	2.2 ± 1.0
C _{max}	(ng/ml)	3.9 ± 1.8	18.1 ± 6.4	3.4 ± 0.8
AUC _{0-∞}	(ng·hr/ml)	N.A.	182.2 ± 70.2	N.A.
Day 5				
T _{max}	(hr)	1.5 ± 0.6	2.3 ± 0.8	1.8 ± 0.4
C _{max}	(ng/ml)	3.3 ± 1.2	18.3 ± 6.0	2.9 ± 0.9
AUC _{0-∞}	(ng·hr/ml)	N.A.	206.6 ± 61.2*	N.A.
Day 10				
T _{max}	(hr)	1.7 ± 0.5	2.2 ± 1.0	1.8 ± 0.4
C _{max}	(ng/ml)	4.6 ± 1.3	19.9 ± 9.5	3.5 ± 1.5
AUC _{0-∞}	(ng·hr/ml)	N.A.	209.4 ± 72.8*	N.A.

Data are expressed as the mean ± S.D.

"N.A." means statistics were not applicable.

* AUC_{0-24} .

747 administrations of 30 and 75 mg, and the inhibition lasted for at least 48 hr. This onset time coincided with the T_{max} of the metabolites of CS-747 (the average T_{max} at single doses of 30 and 75 mg were between 0.6 and 1.5 hr). When compared with the published clinical data for clopidogrel²⁸⁾, these results show that CS-747 was

a more potent antiaggregatory agent with a more rapid onset. The platelet aggregation activity in both of these dose groups returned to the baseline, 7 days after administration. These results are consistent with the contention that CS-747, when orally administered, is rapidly absorbed and converted to the active

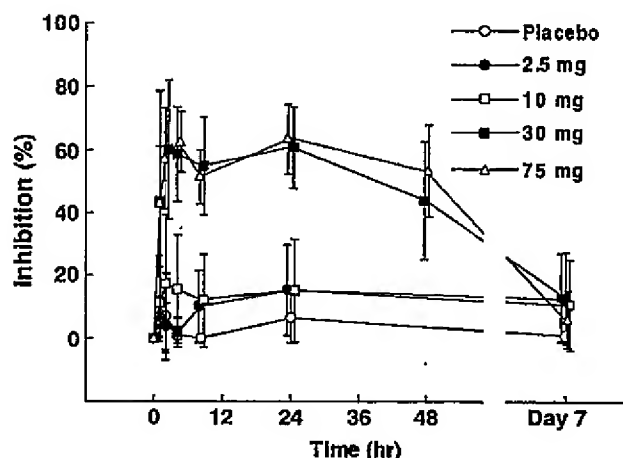


Fig. 4. Inhibition of *ex vivo* platelet aggregation induced by 20 μ M ADP after single administrations of CS-747 (mean \pm S.D.)

metabolite, followed by immediate blocking of the ADP receptors on human platelets. Despite rapid elimination of the metabolites from the plasma, a significant inhibition of platelet aggregation, which lasted, at least, up to 48 hr after the administration was seen. This suggests that the active metabolite bound irreversibly to the receptors on the platelets.

On the other hand, collagen-induced platelet aggregation was hardly inhibited in any of the dose groups. The difference in the platelet aggregation response to these agonists suggests that the mode of action of CS-747 is specific toward ADP.

ii) Food-effect study

There were no significant differences in the maximum inhibition of platelet aggregation and the duration time of the action between the fed and fasting regimens, whereas the onset time was delayed by

about 2 hr in the fed regimen group (Fig. 5). This delay coincided with the delay of the T_{max} in the fed regimen (Table 3).

iii) Multiple dose study

When 10 mg of CS-747 was given once daily, ADP-induced platelet aggregation was significantly and continuously inhibited, by 50 to 80%, starting 2 days after the first administration, and lasting at least until 2 days after the final administration (Fig. 6). When compared with the multiple administration of clopidogrel given at a dose of 75 mg once daily,²⁹⁾ the steady-state inhibition by CS-747 was reached much sooner, and the effect was more potent. The 2.5-mg dose of CS-747 was not associated with a significant inhibition of platelet aggregation. Although the 10-mg single administration of CS-747 was not effective in inhibiting platelet aggregation, the 3-day consecutive administration

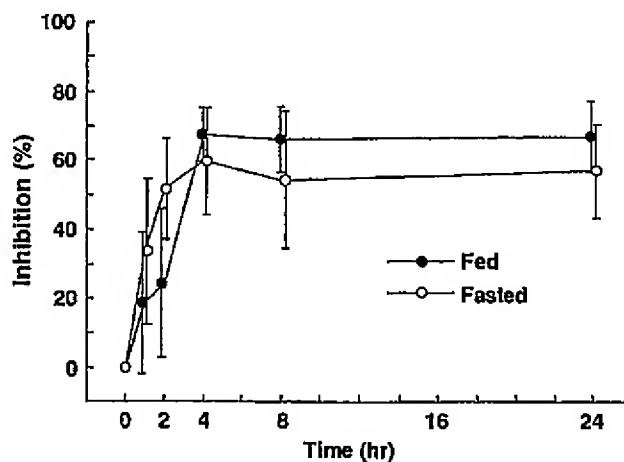


Fig. 5. Effect of food on the inhibition of *ex vivo* platelet aggregation after a single administration of CS-747 at a dose of 25 mg (mean \pm S.D.)

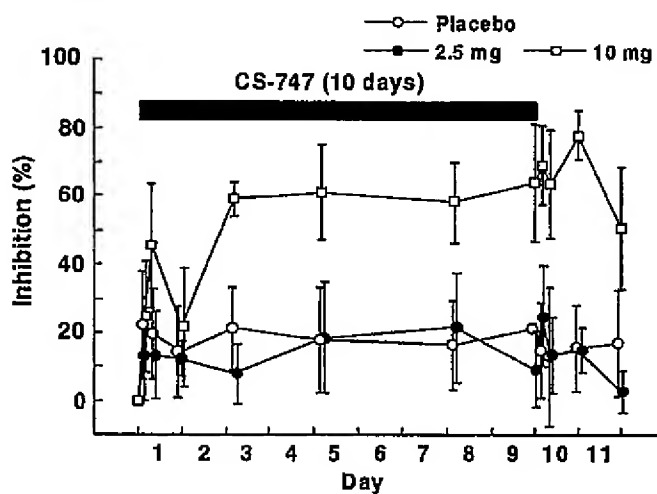


Fig. 6. Inhibition of *ex vivo* platelet aggregation induced by 20 mM ADP after multiple administrations of CS-747 (mean \pm S.D.)

of 10 mg CS-747 caused a more than 50% inhibition. However, no accumulation of any of the metabolites analyzed was observed at all.

3) Prolongation of bleeding time

Inhibition of the ADP-induced platelet aggregation was accompanied by a pro-

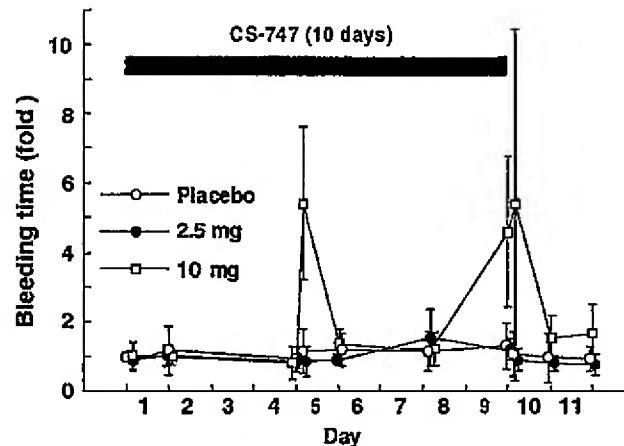


Fig. 7. Prolongation of bleeding time after multiple administrations of CS-747 (mean ± S.D.)

longation of bleeding time in the single and multiple dose studies. At single doses of 30 and 75 mg, and at multiple doses of 10 mg, the prolongation was observed sporadically in some volunteers soon after the administrations. Thus, the effect did not last as long as the inhibition of platelet aggregation. The maximal prolongation of bleeding was less than 6 times the baseline values (Fig. 7) *i.e.* less than 20 min.

3. Safety and Tolerability

CS-747 was well tolerated at single administrations up to 75 mg, and at multiple administrations up to 10 mg. At a dose of 10 mg in the single-dose study, including the food-effect study, one subject complained of a mild headache which was possibly related to the drug. In the multiple-dose study, several events were observed in association with the inhibition of platelet aggregation. All the other events which occurred were considered

either unlikely to be related or not related to the drug. There were no abnormal changes in the safety parameters (see Protocol) in any of the dose groups. All adverse events observed in these studies are shown in Table 5.

4. Conclusion

CS-747 was demonstrated to have a potent and long-lasting inhibitory activity on *ex vivo* platelet aggregation in healthy Caucasian male volunteers, without any serious adverse events. The pharmacodynamic profile of CS-747 as an antiplatelet agent is likely to result from its rapid absorption and rapid formation of the active metabolite, which irreversibly blocks ADP-mediated platelet aggregation.

(Takashi Hirota)

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Table 5. All adverse events observed in the studies

Study /Dose	Event	Severity ^a	Relationship ^b	Study /Dose	Event	Severity ^a	Relationship ^b
Single-dose				Multiple-dose			
Placebo	Dizziness	1	1	Placebo	Application site reaction (2 cases)	1	1
	Nausea	1	1		Headache	2	3
10 mg	Pallor	2	1		Headache	2	2
	Increased sweating	2	1		Dizziness	1	1
	Vomiting	1	1		Nausea	1	1
	Dizziness	3	1		Oral hemorrhage	1	3
	Headache	1	3		Pharyngitis	1	1
	Rhinitis	1	1		Sinusitis	1	1
	Others ^c	1	1		Sinusitis	2	1
30 mg	Dizziness	1	2		Rash	1	2
75 mg	Dizziness	2	1		Conjunctivitis	1	1
	Flatulence	1	2	2.5 mg	Headache	1	3
	Vomiting	1	1		Paraesthesia	1	1
Food-effect				10 mg	Application site reaction (2 cases)	1	1
25 mg	Pallor	1	1		Cellulitis	1	1
	Skin cold and clammy	1	1		Injection site reaction	1	1
	Hot flushes	1	1		Face edema	1	1
	Dizziness	1	1		Oral hemorrhage	1	3
^a Severity is rated on a 4-point scale of increasing severity (Grade 1 to Grade 4).					Increased coagulation time	1	4
^b Relationship to study drug:					Rash	1	1
1: Not related, 2: Unlikely, 3: Possibly, 4: Probably, 5: Definitely.				^c Superficial cuts on scalp frontal area caused by hitting head while fainting.			

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Exhibit 4



US005288726A

United States Patent [19]

Koike et al.

[11] Patent Number: 5,288,726

[45] Date of Patent: Feb. 22, 1994

[54] **TETRAHYDROTHIENOPYRIDINE
DERIVATIVES, FURO AND PYRROLO
ANALOGS THEREOF AND THEIR
PREPARATION AND USES FOR
INHIBITING BLOOD PLATELET
AGGREGATION**

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[52] U.S. Cl. 514/301; 546/114

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514/302

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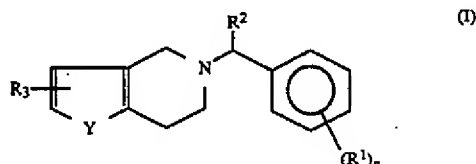
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[57] ABSTRACT

Compounds of formula (I):



wherein: R¹ is hydrogen, alkyl, halogen, haloalkyl, hydroxy, alkoxy, haloalkoxy, alkylthio, haloalkylthio, amino, alkanoyl, haloalkanoyl, carboxy, alkoxy-carbonyl, carbamoyl, cyano, nitro, alkanesulfonyl, haloalkanesulfonyl or sulfamoyl; R² is optionally substituted alkanoyl, optionally substituted alkenoyl, optionally substituted cycloalkylcarbonyl, substituted benzoyl, or 5,6-dihydro-1,4,2-dioxazin-3-yl; R³ is hydrogen, hydroxy, optionally substituted alkoxy, aralkyloxy, alkanoyloxy, alkenoyloxy, cycloalkylcarbonyloxy, aryl-carbonyloxy, alkoxy-carbonyloxy, aralkyloxy-carbonyloxy, phthalidylloxy, (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy, (5-phenyl-2-oxo-1,3-dioxolen-4-yl)methoxy, optionally substituted amino or nitro; Y is —NH— or oxygen or sulfur; n is 1 to 5; and tautomers and salts of said compounds of formula (I), have the ability to inhibit blood platelet aggregation, and can thus be used for treatment and prophylaxis of thrombosis and embolisms.

56 Claims, No Drawings

**TETRAHYDROTHIENOPYRIDINE
DERIVATIVES, FURO AND PYRROLO ANALOGS
THEREOF AND THEIR PREPARATION AND
USES FOR INHIBITING BLOOD PLATELET
AGGREGATION**

BACKGROUND OF THE INVENTION

The present invention relates to a series of new tetrahydrothieno[3,2-c]pyridine derivatives and furo and pyrrolo analogs of these derivatives, and provides processes for preparing these derivatives as well as methods and compositions using them for inhibiting blood platelet aggregation.

A number of tetrahydrothienopyridine and tetrahydrofurofuro derivatives is known, and some of these have been disclosed to have the ability to inhibit blood platelet aggregation. For example, U.S. Pat. Nos. 4,051,141, 4,075,215, 4,127,580, 4,464,377 and 4,529,596 all disclose compounds of this type, although not all disclose them for the inhibition of blood platelet aggregation. The closest prior art is believed to be U.S. Pat. No. 4,051,141, which discloses, inter alia, 5-(2-chlorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and U.S. Pat. No. 4,529,596, which discloses, inter alia, 5-(2-chloro- α -methoxycarbonylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine.

However, there are problems with the prior art compounds referred to above, especially in that many of them require a long time after administration before they manifest their activity. Accordingly, there is a need for new compounds of this type having improved activity and the ability to act faster.

We have now discovered a series of new tetrahydrothieno[3,2-c]pyridine derivatives and furo and pyrrolo analogs of these derivatives which have an improved ability to inhibit the aggregation of blood platelets.

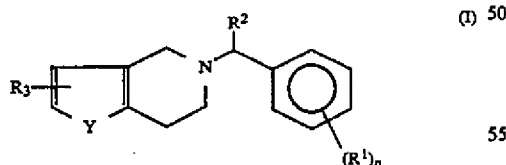
BRIEF SUMMARY OF INVENTION

It is, therefore, an object of the present invention to provide a series of new compounds of this type.

It is a further, and more specific object of the present invention to provide such compounds having valuable inhibitory activity against platelet aggregation.

Other objects and advantages of the present invention will become apparent as the description proceeds.

The compounds of the present invention are those compounds of formula (I):



wherein:

R^1 represents a hydrogen atom, an alkyl group having from 1 to 4 carbon atoms, a haloalkyl group having from 1 to 4 carbon atoms and at least one halogen atom, a hydroxy group, an alkoxy group having from 1 to 4 carbon atoms, a haloalkoxy group having from 1 to 4 carbon atoms and at least one halogen atom, an alkylthio group having from 1 to 4 carbon atoms, a haloalkylthio group having from 1 to 4 carbon atoms and at least one halogen atom, an amino group, an alkanoyl

group having from 1 to 5 carbon atoms, a haloalkanoyl group having from 2 to 5 carbon atoms and at least one halogen atom, a carboxy group, an alkoxycarbonyl group having from 2 to 5 carbon atoms, a carbamoyl group, a cyano group, a nitro group, an alkanesulfonyl group having from 1 to 4 carbon atoms, a haloalkanesulfonyl group having from 1 to 4 carbon atoms and at least one halogen atom, or a sulfamoyl group;

R^2 represents an alkanoyl group having from 1 to 10 carbon atoms, a substituted alkanoyl group which has from 2 to 10 carbon atoms and which is substituted by at least one substituent selected from the group consisting of substituents A, defined below, an alkenoyl group having from 3 to 6 carbon atoms, a substituted alkenoyl group which has from 3 to 6 carbon atoms and which is substituted by at least one substituent selected from the group consisting of substituents A, defined below, a cycloalkylcarbonyl group having from 4 to 8 carbon atoms, a substituted cycloalkylcarbonyl group which has from 4 to 8 carbon atoms and which is substituted by at least one substituent selected from the group consisting of substituents A, defined below, a substituted benzoyl group having at least one substituent selected from the group consisting of substituents B, defined below, or a 5,6-dihydro 1,4,2-dioxazin-3-yl group;

R^3 represents a hydrogen atom, a hydroxy group, an alkoxy group having from 1 to 4 carbon atoms, a substituted alkoxy group which has from 1 to 4 carbon atoms and which is substituted by at least one substituent selected from the group consisting of substituents C, defined below, an aralkyloxy group in which the aralkyl part is as defined below, an alkanoyloxy group having from 1 to 18 carbon atoms, an alkenoyloxy group having from 3 to 6 carbon atoms, a cycloalkyl carbonyloxy group having from 4 to 8 carbon atom, an arylcarbonyloxy group in which the aryl part is as defined below, an alkoxycarbonyloxy group having from 2 to 5 carbon atoms, an aralkyloxycarbonyloxy group in which the aralkyl part is as defined below, a phthalidyloxy group, a (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy group, a (5-phenyl-2-oxo-1,3-dioxolen-4-yl)methoxy group, a group of formula $-NR^aR^b$

wherein R^a and R^b are independently selected from the group consisting of hydrogen atoms, alkyl groups having from 1 to 4 carbon atoms and substituted alkyl groups which have from 1 to 4 carbon atoms and which are substituted by at least one substituent selected from the group consisting of substituents C, defined below,

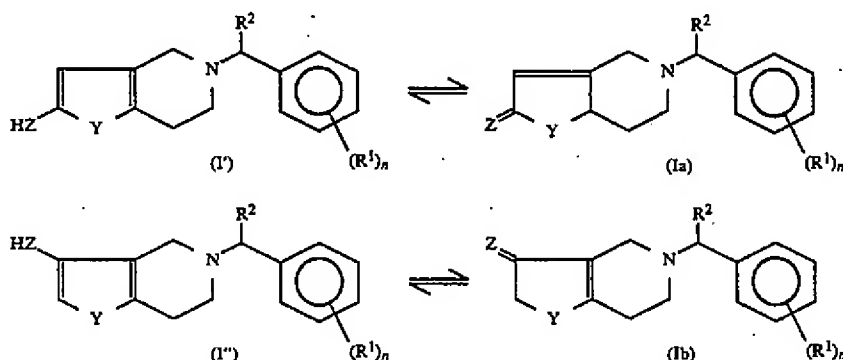
an aralkylamino group in which the aralkyl part is as defined below, an alkanoylamino group having from 1 to 18 carbon atoms, an alkenoylamino group having from 3 to 6 carbon atoms, a cycloalkylcarbonylamino group having from 4 to 8 carbon atoms, an arylcarbonylamino group in which the aryl part is as defined below, an alkoxycarbonylamino group having from 2 to 5 carbon atoms, an aralkyloxycarbonylamino group in which the aralkyl part is as defined below, a phthalidylamino group, a (5-methyl-2-oxo-1,3-dioxolen-4-yl)methylamino group, a (5-phenyl-2-

oxo-1,3-dioxolen-4-yl)methylamino group or a nitro group;
 Y represents a group of formula —NH— or an oxygen or sulfur atom; and
 n is an integer from 1 to 5, and, when n is an integer from 2 to 5, the groups represented by R^1 may be the same as or different from each other;
 said substituents A are selected from the group consisting of halogen atoms, hydroxy groups, alkoxy groups having from 1 to 4 carbon atoms and cyano

The invention also provides processes for preparing these compounds, which are described in greater detail hereafter.

DETAILED DESCRIPTION OF INVENTION

When the compounds of the present invention have an amino or hydroxy group at the 2- or 3- position (i.e. R^3 represents an amino or hydroxy group at the 2- or 3- position), they can exist as keto-enol tautomers, that is:



groups;
 said substituents B are selected from the group consisting of alkoxy groups having from 1 to 4 carbon atoms, halogen atoms and alkoxy groups having from 1 to 4 carbon atoms;
 said substituents C are selected from the group consisting of alkoxy groups having from 1 to 4 carbon atoms, alkanoyloxy groups having from 1 to 6 carbon atoms and arylcarbonyloxy groups in which the aryl part is as defined below;
 said aralkyl parts of said aralkyloxy, aralkyloxy, carbonyloxy, aralkylamino and aralkyloxycarbonylamino groups are alkyl groups which have from 1 to 4 carbon atoms and which are substituted by at least one aryl group as defined below;
 said aryl groups and said aryl parts of said arylcarbonyloxy groups and of said arylcarbonylamino groups have from 6 to 10 carbon atoms in a carbocyclic ring which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D, defined below; and
 said substituents D are selected from the group consisting of the groups and atoms defined above in relation to R^1 , other than said hydrogen atom;
 and tautomers thereof and pharmaceutically acceptable salts of said compounds of formula (I) and of said tautomers.

The invention also provides a pharmaceutical composition for the treatment and prophylaxis of thrombosis or embolisms, comprising an effective amount of a blood platelet aggregation inhibitor in admixture with a pharmaceutically acceptable carrier or diluent, wherein said inhibitor is at least one compound of formula (I), or a tautomer or pharmaceutically acceptable salt thereof.

The invention still further provides a method for the treatment or prophylaxis of thrombosis or embolisms, comprising administering to a mammal, which may be human, an effective amount of a blood platelet aggregation inhibitor, wherein said inhibitor is at least one compound of formula (I), or a tautomer or pharmaceutically acceptable salt thereof.

wherein Y, R^1 , R^2 and n are as defined above, and Z represents a group of formula =NH or an oxygen atom. These tautomers may or may not be readily separable, and, if separable, may be separated by methods well known in the art. In any event, the present invention embraces both the individual isolated tautomers, as well as mixtures thereof, and both the isolated tautomers and such mixtures may be used in the compositions and methods of the present invention. In particular, the tautomers of formula (Ia) are preferred.

In the compounds of the present invention, where R^1 represents an alkyl group, this may be a straight or branched chain alkyl group having from 1 to 4 carbon atoms, and examples include the methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl and t-butyl groups. Of these, we prefer those alkyl groups having from 1 to 3 carbon atoms, more preferably the methyl and ethyl groups.

Where R^1 represents a halogen atom, this may be, for example, a fluorine, chlorine, iodine or bromine atom, and is preferably a fluorine or chlorine atom.

Where R^1 represents a haloalkyl group, the alkyl part may be any one of the alkyl groups exemplified above and may be substituted by one or more halogen (for example fluorine, chlorine, bromine or iodine) atoms. There is, in principle, no restriction on the number of halogen substituents on the alkyl group, this being limited only by the number of substitutable atoms. In general, however, from 1 to 5 halogen substituents are preferred, from 1 to 3 substituents being more preferred. Specific examples of such groups include the fluoromethyl, difluoromethyl, trifluoromethyl, chloromethyl, dichloromethyl, trichloromethyl, 2-fluoroethyl, 2-chloroethyl, 2-bromoethyl, 2-iodoethyl, 2,2,2-trichloroethyl, 2,2,2-trifluoroethyl, 2-fluoropropyl, 3-fluoropropyl, 3-chloropropyl, 2-fluorobutyl, 3-fluorobutyl, 4-chlorobutyl and 4-fluorobutyl groups. The fluorine-substituted and chlorine-substituted groups are preferred, the fluorine-substituted groups being more preferred. The fluoromethyl, difluoromethyl and trifluoromethyl

groups are most preferred, especially the trifluoromethyl group.

Where R^1 represents an alkoxy group, this may be a straight or branched chain alkoxy group having from 1 to 4 carbon atoms, and examples include the methoxy, ethoxy, propoxy, isopropoxy, butoxy, isobutoxy, sec-butoxy and t-butoxy groups. Of these, we prefer those alkoxy groups having from 1 to 3 carbon atoms, more preferably the methoxy and ethoxy groups.

Where R^1 represents a haloalkoxy group, the alkoxy part may be any one of the alkoxy groups exemplified above and may be substituted by one or more halogen (for example fluorine, chlorine, bromine or iodine) atoms. There is, in principle, no restriction on the number of halogen substituents on the alkoxy group, this being limited only by the number of substitutable atoms. In general, however, from 1 to 5 halogen substituents are preferred, from 1 to 3 substituents being more preferred. Specific examples of such groups include the fluoromethoxy, difluoromethoxy, trifluoromethoxy, 2-fluoroethoxy, 2-chloroethoxy, 2-bromoethoxy, 2-iodoethoxy, 2,2,2-trichloroethoxy, 2,2,2-trifluoroethoxy, 2-fluoropropoxy, 3-fluoropropoxy, 3-chloropropoxy, 2-fluorobutoxy, 3-fluorobutoxy, 4-chlorobutoxy and 4-fluorobutoxy groups. The fluoroalkoxy groups are preferred. The fluoromethoxy, difluoromethoxy and trifluoromethoxy groups are most preferred, especially the trifluoromethoxy group.

Where R^1 represents an alkylthio group, this may be a straight or branched chain alkylthio group having from 1 to 4 carbon atoms, and examples include the methylthio, ethylthio, propylthio, isopropylthio, butylthio, isobutylthio, sec-butylthio and t-butylthio groups. Of these, we prefer those alkylthio groups having from 1 to 3 carbon atoms, more preferably the methylthio and ethylthio groups.

Where R^1 represents a haloalkylthio group, the alkylthio part may be any one of the alkylthio groups exemplified above and may be substituted by one or more halogen (for example fluorine, chlorine, bromine or iodine) atoms. There is, in principle, no restriction on the number of halogen substituents on the alkylthio group, this being limited only by the number of substitutable atoms. In general, however, from 1 to 5 halogen substituents are preferred, from 1 to 3 substituents being more preferred. Specific examples of such groups include the fluoromethylthio, difluoromethylthio, trifluoromethylthio, 2-fluoroethylthio, 2-chloroethylthio, 2-bromoethylthio, 2-iodoethylthio, 2,2,2-trichloroethylthio, 2,2,2-trifluoroethylthio, 2-fluoropropylthio, 3-fluoropropylthio, 3-chloropropylthio, 2-fluorobutylthio, 3-fluorobutylthio, 4-chlorobutylthio and 4-fluorobutylthio groups. The fluorine substituted groups are preferred. The fluoromethylthio, difluoromethylthio and trifluoromethylthio groups are most preferred, especially the trifluoromethylthio group.

Where R^1 represents an alkanoyl group, this has from 1 to 5 carbon atoms and may be a straight or branched chain group. Examples include the formyl, acetyl, propionyl, butyryl, isobutyryl, valeryl, isovaleryl and pivaloyl groups, of which the formyl and acetyl groups are preferred.

Where R^1 represents a haloalkanoyl group, this has from 2 to 5 carbon atoms and may be a straight or branched chain group. Examples include the fluoroacetyl, difluoroacetyl, trifluoroacetyl, chloroacetyl, trichloroacetyl, bromoacetyl, iodoacetyl, 3-fluoropropionyl, 4-fluorobutyryl and 5-fluorovaleryl groups. Of

these, the fluorine substituted alkanoyl groups are preferred, the fluoroacetyl, difluoroacetyl and trifluoroacetyl groups being more preferred and the trifluoroacetyl group being most preferred.

Where R^1 represents an alkoxycarbonyl group, this may be a straight or branched chain alkoxycarbonyl group having from 2 to 5 carbon atoms, that is the alkoxy part has from 1 to 4 carbon atoms, and examples include the methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, isopropoxycarbonyl, butoxycarbonyl, isobutoxycarbonyl, sec-butoxycarbonyl and t-butoxycarbonyl groups. Of these, we prefer those alkoxycarbonyl groups having from 1 to 3 carbon atoms, more preferably the methoxycarbonyl and ethoxycarbonyl groups.

Where R^1 represents an alkanesulfonyl group, this may be a straight or branched chain alkanesulfonyl group having from 1 to 4 carbon atoms, and examples include the methanesulfonyl, ethanesulfonyl, propanesulfonyl, isopropanesulfonyl, butanesulfonyl, isobutanesulfonyl, sec butanesulfonyl and t-butanesulfonyl groups. Of these, we prefer those alkanesulfonyl groups having from 1 to 3 carbon atoms, more preferably the methanesulfonyl and ethanesulfonyl groups.

Where R^1 represents a haloalkanesulfonyl group, the alkanesulfonyl part may be any one of the alkanesulfonyl groups exemplified above and may be substituted by one or more halogen (for example fluorine, chlorine, bromine or iodine) atoms. There is, in principle, no restriction on the number of halogen substituents on the alkanesulfonyl group, this being limited only by the number of substitutable atoms. In general, however, from 1 to 5 halogen substituents are preferred, from 1 to 3 substituents being more preferred. Specific examples of such groups include the fluoromethanesulfonyl, difluoromethanesulfonyl, trifluoromethanesulfonyl, dichloromethanesulfonyl, trichloromethanesulfonyl, 2-fluoroethanesulfonyl, 2-chloroethanesulfonyl, 2-bromoethanesulfonyl, 2-iodoethanesulfonyl, 2,2,2-trichloroethanesulfonyl, 2,2,2-trifluoroethanesulfonyl, 2-fluoropropanesulfonyl, 3-fluoropropanesulfonyl, 3-chloropropanesulfonyl, 2-fluorobutanesulfonyl, 3-fluorobutanesulfonyl, 4-chlorobutanesulfonyl and 4-fluorobutanesulfonyl groups. The fluorine-substituted alkanesulfonyl and chlorine substituted alkanesulfonyl groups are preferred, the fluorine-substituted alkanesulfonyl groups being more preferred. The fluoromethanesulfonyl, difluoromethanesulfonyl and trifluoromethanesulfonyl groups are most preferred, especially the trifluoromethanesulfonyl group.

Of the above groups and atoms, we especially prefer that R^1 should represent: a hydrogen atom; an alkyl group having from 1 to 4 carbon atoms; a halogen atom; a fluorine substituted alkyl group having from 1 to 4 carbon atoms; a hydroxy group; an alkoxy group having from 1 to 4 carbon atoms; a fluorine-substituted alkoxy group having from 1 to 4 carbon atoms; an alkylthio group having from 1 to 4 carbon atoms; a fluorine-substituted alkylthio group having from 1 to 4 carbon atoms; an amino group; an alkanoyl group having from 1 to 5 carbon atoms; a fluorine-substituted alkanoyl group having from 2 to 5 carbon atoms; an alkoxycarbonyl group having from 2 to 5 carbon atoms; a carbamoyl group; a cyano group; a nitro group; an alkanesulfonyl group having from 1 to 4 carbon atoms; a fluorine-substituted alkanesulfonyl group having from 1 to 4 carbon atoms; or a sulfamoyl group.

More preferably R^1 represents: a hydrogen atom; a methyl group; an ethyl group; a halogen atom; a fluo-

rine-substituted methyl group; a hydroxy group; a methoxy group; an ethoxy group; a fluorine-substituted methoxy group; a methylthio group; a fluorine-substituted methylthio group; a formyl group; an acetyl group; a fluorine-substituted acetyl group; a methoxycarbonyl group; an ethoxycarbonyl group; a propoxycarbonyl group; a carbamoyl group; a cyano group; a nitro group; a methanesulfonyl group; an ethanesulfonyl group; a fluorine-substituted methanesulfonyl group; or a sulfamoyl group.

Still more preferably R^1 represents: a halogen atom; a trifluoromethyl group; a hydroxy group; a difluoromethoxy group; a trifluoromethoxy group; a difluoromethylthio group; a trifluoromethylthio group; a formyl group; an acetyl group; a trifluoroacetyl group; a cyano group or a nitro group.

Most preferably R^1 represents: a fluorine atom, a chlorine atom or a trifluoromethyl group; especially a fluorine atom or a chlorine atom.

The number of the substituents, n , represented by R^1 is from 1 to 5, although the maximum may be lower than 5 in some cases if there is a problem of steric hindrance. Preferably n is from 1 to 3, and more preferably 1 or 2. The position of substitution by R^1 on the phenyl group is preferably para or ortho, more preferably ortho.

Where R^2 represents an alkanoyl group having from 1 to 10 carbon atoms, this may be a straight or branched chain group, and examples include the formyl, acetyl, propionyl, butyryl, isobutyryl, valeryl, isovaleryl, pivaloyl, hexanoyl, heptanoyl, octanoyl, nonanoyl and decanoyl groups, of which those groups having from 2 to 6 carbon atoms are preferred, especially the acetyl, propionyl and isobutyryl groups, of which the acetyl and propionyl groups are most preferred.

Those alkanoyl groups represented by R^2 and having from 2 to 10 carbon atoms may be substituted by one or more of substituents A, defined above. Examples of such substituents A include:

halogen atoms, such as the fluorine, chlorine, bromine and iodine atoms;

hydroxy groups;

alkoxy groups having from 1 to 4 carbon atoms, such as those exemplified above in relation to R^1 ; and cyano groups.

In the case of these substituted groups, and all substituted groups referred to herein, there is no specific limitation on the number of the substituents, except such as may be imposed by the number of substitutable positions and possibly also by steric constraints. However, in general, from 1 to 3 such substituents are preferred.

Specific examples of such substituted alkanoyl groups include the fluoroacetyl, difluoroacetyl, trifluoroacetyl, chloroacetyl, trichloroacetyl, bromoacetyl, iodoacetyl, 3-fluoropropionyl, 3-chloropropionyl, 3-bromopropionyl, 3-iodopropionyl, 4-fluorobutyryl, 4-chlorobutyryl, 5-fluorovaleryl, hydroxyacetyl, 3-hydroxypropionyl, 4-hydroxybutyryl, 5-hydroxyvaleryl, methoxyacetyl, 3-methoxypropionyl, 4-methoxybutyryl, 5-methoxyvaleryl, ethoxyacetyl, 3-ethoxypropionyl, 4-ethoxybutyryl, 5-ethoxyvaleryl, cyanoacetyl, 3-cyanopropionyl, 4-cyanobutyryl and 5-cyanovaleryl groups, of which the fluoroacetyl, difluoroacetyl, trifluoroacetyl, chloroacetyl, 3-fluoropropionyl, 3-chloropropionyl, hydroxyacetyl, 3-hydroxypropionyl, methoxyacetyl, 3-methoxypropionyl, ethoxyacetyl, cyanoacetyl and 3-cyanopropionyl groups are more preferred. Still more preferred are the fluoroacetyl, difluoroacetyl, trifluoro-

acetyl, chloroacetyl, 3-fluoropropionyl, hydroxyacetyl, methoxyacetyl, ethoxyacetyl and cyanoacetyl groups. The most preferred groups are the fluoroacetyl, difluoroacetyl, trifluoroacetyl, chloroacetyl, 3-fluoropropionyl, hydroxyacetyl, methoxyacetyl and cyanoacetyl groups, especially the fluoroacetyl, difluoroacetyl and trifluoroacetyl groups.

Where R^2 represents an alkenoyl group having from 3 to 6 carbon atoms, this may be a straight or branched chain group, and examples include the acryloyl, methacryloyl, 2-butenoyl, 2-pentenoyl and 2-hexenoyl groups, of which the acryloyl and methacryloyl groups are preferred.

These alkenoyl groups may also be substituted by one or more of substituents A, defined and exemplified above. Specific examples of such substituted groups include the 3-fluoroacryloyl, 3-chloroacryloyl and 3-cyanoacryloyl groups, of which the 3-fluoroacryloyl group is particularly preferred.

Where R^2 represents a cycloalkylcarbonyl group, this has from 4 to 8 carbon atoms, that is the cycloalkyl group itself has from 3 to 7 ring carbon atoms. Examples of such groups include the cyclopropylcarbonyl, cyclobutylcarbonyl, cyclopentylcarbonyl, cyclohexylcarbonyl and cycloheptylcarbonyl groups, of which the cyclopropylcarbonyl and cyclobutylcarbonyl groups are particularly preferred.

These cycloalkylcarbonyl groups may also be substituted by one or more of substituents A, defined and exemplified above. Specific examples of such substituted groups include the 2-fluorocyclopropylcarbonyl, 2,2-difluorocyclopropylcarbonyl, 2-chlorocyclopropylcarbonyl, 2-bromocyclopropylcarbonyl, 2-fluorocyclobutylcarbonyl, 2-chlorocyclobutylcarbonyl, 2-fluorocyclopentylcarbonyl, 2-chlorocyclopentylcarbonyl, 2-fluorocyclohexylcarbonyl, 2-chlorocyclohexylcarbonyl, 2-hydroxycyclopropylcarbonyl, 2-hydroxycyclobutylcarbonyl, 2-hydroxycyclopentylcarbonyl, 2-hydroxycyclohexylcarbonyl, 2-methoxycyclopropylcarbonyl, 2-methoxycyclobutylcarbonyl, 2-methoxycyclopentylcarbonyl, 2-methoxycyclohexylcarbonyl, 2-ethoxycyclopropylcarbonyl, 2-ethoxycyclobutylcarbonyl, 2-ethoxycyclopentylcarbonyl, 2-ethoxycyclohexylcarbonyl, 2-cyanocyclopropylcarbonyl, 2-cyanocyclobutylcarbonyl, 2-cyanocyclopentylcarbonyl and 2-cyanocyclohexylcarbonyl groups, of which the 2-fluorocyclopropylcarbonyl, 2,2-difluorocyclopropylcarbonyl, 2-chlorocyclopropylcarbonyl, 2-fluorocyclobutylcarbonyl, 2-chlorocyclobutylcarbonyl, 2-fluorocyclopentylcarbonyl, 2-fluorocyclohexylcarbonyl, 2-hydroxycyclopropylcarbonyl, 2-methoxycyclopropylcarbonyl, 2-ethoxycyclopropylcarbonyl and 2-cyanocyclopropylcarbonyl groups are preferred. More preferred groups are the 2-fluorocyclopropylcarbonyl, 2-chlorocyclopropylcarbonyl, 2-fluorocyclobutylcarbonyl and 2-methoxycyclopropylcarbonyl groups, and the most preferred is the 2-fluorocyclopropylcarbonyl group.

Where R^2 represents a substituted benzoyl group, this is substituted by at least one of substituents B, which are selected from the group consisting of alkyl groups having from 1 to 4 carbon atoms, halogen atoms and alkoxy groups having from 1 to 4 carbon atoms, all of which may be as exemplified in relation to the same groups and atoms represented by R^1 . The number of the substituents may be from 1 to 5, provided that there is no problem of steric hindrance; preferably, however, are from 1 to 3 substituents, more preferably 1 or Specific exam-

ples of such substituted benzoyl groups include the 2-fluorobenzoyl, 3-fluorobenzoyl, 4-fluorobenzoyl, 2,4-difluorobenzoyl, 2,4,6-trifluorobenzoyl, 2,3,4,5,6-pentafluorobenzoyl, 4-chlorobenzoyl, 2,4-dichlorobenzoyl, 4-methylbenzoyl, 2,4-dimethylbenzoyl, 4-ethylbenzoyl, 2,4-diethylbenzoyl, 4-methoxybenzoyl, 2,4-dimethoxybenzoyl, 4-ethoxybenzoyl and 2,4-diethoxybenzoyl groups, of which the 4-fluorobenzoyl and 2,4-difluorobenzoyl groups are preferred.

Where R^3 represents an alkoxy group, this may be a straight or branched chain group having from 1 to 4 carbon atoms and may be any of the alkoxy groups exemplified above in relation to R^1 . Such a group may be unsubstituted or it may have one or more substituents selected from the group consisting of substituents C,

defined above, and examples of which are as follows: alkoxy groups having from 1 to 4 carbon atoms, such as those exemplified above in relation to R^1 ;

alkanoyloxy groups having from 1 to 6 carbon atoms, which may be a straight or branched chain group, for example the formyloxy, acetoxy, propionyloxy, butyryloxy, isobutyryloxy, valeryloxy, isovaleryloxy, pivaloyloxy or hexanoyloxy groups, of which those groups having from 1 to 5 carbon atoms are preferred, and the acetoxy, propionyloxy, butyryloxy and pivaloyloxy groups are most preferred; and

arylcabonyloxy groups in which the aryl part is as defined above, for example the arylcabonyloxy groups exemplified below in relation to R^3 .

Specific examples of such substituted alkoxy groups include the methoxymethoxy, ethoxymethoxy, propoxymethoxy, butoxymethoxy, 2-methoxyethoxy, 2-ethoxyethoxy, formyloxymethoxy, acetoxymethoxy, propionyloxymethoxy, 2-formyloxyethoxy, 2-acetoxyethoxy, 2-propionyloxyethoxy, 3-acetoxypropoxy, 4-acetoxybutoxy, valeryloxymethoxy, pivaloyloxymethoxy, benzoyloxymethoxy, naphthoyloxymethoxy, p-toluoyloxymethoxy, p-chlorobenzoyloxymethoxy, 2-benzoyloxyethoxy, 3-benzoyloxypropoxy and 4-benzoyloxybutoxy groups, of which the pivaloyloxymethoxy group is most preferred.

Where R^3 represents an aralkyloxy group, the alkoxy part is an alkoxy group having from 1 to 4, preferably from 1 to 3, carbon atoms, such as those exemplified above in relation to R^1 , especially the methoxy, ethoxy, propoxy or isopropoxy groups. The aryl part is as defined above and has from 6 to 10, preferably 6 or 10, ring carbon atoms. Examples of such aryl groups include the phenyl, 1-naphthyl and 2-naphthyl groups and such groups which are substituted by one or more of substituents D, defined above and examples of which have been given in relation to the same groups and atoms which may be represented by R^1 . The alkoxy part may be substituted by one or more aryl groups, the maximum being dictated only by the number of substitutable positions and possibly also by steric constraints; however, from 1 to 3 aryl groups are normally preferred, 1 or 2 being more preferred and 1 being most preferred. Specific examples of the aralkyloxy groups include the benzyloxy, 1-naphthylmethoxy, 2-naphthylmethoxy, phenethylloxy, α -methylbenzyloxy, 3-phenylpropoxy, 2-phenylpropoxy, 1-phenylpropoxy, 4-phenylbutoxy, benzhydryloxy (i.e. diphenylmethoxy) and trityloxy (i.e. triphenylmethoxy) groups (of these, the benzyloxy and phenethylloxy groups are preferred), and such groups which are substituted by one or more of substituents D.

Where R^3 represents an alkanoyloxy group, this may be a straight or branched chain group and has from 1 to 18 carbon atoms. Examples of such groups include the formyloxy, acetoxy, propionyloxy, butyryloxy, isobutyryloxy, valeryloxy, isovaleryloxy, pivaloyloxy, hexanoyloxy, heptanoyloxy, octanoyloxy, nonanoyloxy, decanoyloxy, lauroyloxy, myristoyloxy, palmitoyloxy and stearoyloxy groups, of which those groups having from 1 to 12 carbon atoms are preferred, those having from 2 to 10 carbon atoms are more preferred, and those having from 2 to 5 carbon atoms are most preferred, especially the acetoxy, propionyloxy, butyryloxy, pivaloyloxy, nonanoyloxy and decanoyloxy groups, of which the acetoxy, propionyloxy, butyryloxy and pivaloyloxy groups are most preferred.

Where R^3 represents an alkenoyloxy group, this may be a straight or branched chain group and has from 3 to 6, more preferably 3 or 4, carbon atoms. Examples of such groups include the acryloyloxy, methacryloyloxy, 2-butenoyloxy, 2-pentenoyloxy and 2-hexenoyloxy groups, of which the acryloyloxy and methacryloyloxy groups are preferred.

Where R^3 represents a cycloalkylcarbonyloxy group, this has from 4 to 8, more preferably from 4 to 7, carbon atoms, that is the cycloalkyl group itself has from 3 to 7 ring carbon atoms. Examples of such groups include the cyclopropylcarbonyloxy, cyclobutylcarbonyloxy, cyclopentylcarbonyloxy, cyclohexylcarbonyloxy and cycloheptylcarbonyloxy groups, of which the cyclopropylcarbonyloxy and cyclobutylcarbonyloxy groups are particularly preferred.

Where R^3 represents an arylcarbonyloxy group, the aryl part is as defined above, and examples of such groups include the benzoyloxy, 1-naphthoyloxy, 2-naphthoyloxy, o-, m- and p-toluoyloxy, o-, m- and p-chlorobenzoyloxy, o-, m- and p-fluorobenzoyloxy, o-, m- and p-methoxybenzoyloxy, 2,3-, 2,4-, 2,5-, 2,6-, 3,4- and 3,5-dichlorobenzoyloxy, 2,4-difluorobenzoyloxy and 2,4,6-trifluorobenzoyloxy groups, preferably the benzoyloxy group.

Where R^3 represents an alkoxycarbonyloxy group, this may be a straight or branched chain alkoxycarbonyloxy group having from 2 to 5 carbon atoms, that is the alkoxy part has from 1 to 4 carbon atoms, and examples include the methoxycarbonyloxy, ethoxycarbonyloxy, propoxycarbonyloxy, isopropoxycarbonyloxy, butoxycarbonyloxy, isobutoxycarbonyloxy, sec-butoxycarbonyloxy and t-butoxycarbonyloxy groups. Of these, we prefer those alkoxycarbonyloxy groups having from 1 to 3 carbon atoms in the alkoxy part and the t-butoxycarbonyloxy group, more preferably the methoxycarbonyloxy, ethoxycarbonyloxy and t-butoxycarbonyloxy groups.

Where R^3 represents an aralkyloxycarbonyloxy group, the alkoxy part is an alkoxy group having from 1 to 4, preferably from 1 to 3, carbon atoms, such as those exemplified above in relation to R^1 , especially the methoxy, ethoxy, propoxy or isopropoxy groups. The aryl part is as defined above and has from 6 to 10, preferably 6 or 10, ring carbon atoms. Examples of such aryl groups include the phenyl, 1-naphthyl and 2-naphthyl groups and such groups which are substituted by one or more of substituents D, defined above and examples of which have been given in relation to the same groups and atoms which may be represented by R^1 . The alkoxy part may be substituted by one or more aryl groups, the maximum being dictated only by the number of substitutable positions and possibly also by steric constraints;

however, from 1 to 3 aryl groups are normally preferred, 1 or 2 being more preferred and 1 being most preferred. Specific examples of the aralkyloxycarbonyloxy groups include the benzyloxycarbonyloxy, 1-naphthylmethoxycarbonyloxy, 2-naphthylmethoxycarbonyloxy, phenethylloxycarbonyloxy, α -methylbenzyloxy carbonyloxy, 3-phenylpropoxycarbonyloxy, 2-phenylpropoxycarbonyloxy, 1-phenylpropoxycarbonyloxy, 4-phenylbutoxycarbonyloxy, benzhydryloxycarbonyloxy and trityloxycarbonyloxy groups (of these, the benzyloxycarbonyloxy group is preferred), and such groups which are substituted by one or more of substituents D.

Where R^3 represents a group of formula $-NR^aR^b$, R^a and R^b are independently selected from the group consisting of hydrogen atoms, alkyl groups having from 1 to 4 carbon atoms and substituted alkyl groups which have from 1 to 4 carbon atoms and which are substituted by at least one substituent selected from the group consisting of substituents C, defined above. Examples of the alkyl groups which may be represented by R^a and R^b are as given above in relation to R^1 , and examples of the substituted alkyl groups which may be represented by R^a and R^b are the substituted alkyl groups corresponding to the substituted alkoxy groups, as given above in relation to R^3 . Specific examples of these groups of formula $-NR^aR^b$ include amino, methylamino, ethylamino, propylamino, isopropylamino, butylamino, isobutylamino, sec-butylamino, t-butylamino, dimethylamino, diethylamino, dipropylamino, diisopropylamino, dibutylamino, methylethylamino, methylpropylamino, N-(methoxymethyl)amino, N-(2-methoxyethyl)amino, N-(acetoxymethyl)amino, N-(pivaloyloxymethyl)amino, N-(benzoylmethyl)amino, N-(2-acetoxyethyl)amino, N-(2-pivaloyloxyethyl)amino and N-(2-benzoyloxyethyl)amino groups, preferably the amino, methylamino, ethylamino, N-(acetoxymethyl)amino and N-(pivaloyloxymethyl)amino groups.

Where R^3 represents an aralkylamino group, the alkyl part is an alkyl group having from 1 to 4, preferably from 1 to 3, carbon atoms, such as those exemplified above in relation to R^1 , especially the methyl, ethyl, propyl or isopropyl groups. The aryl part is as defined above and has from 6 to 10, preferably 6 or 10, ring carbon atoms. Examples of such aryl groups include the phenyl, 1-naphthyl and 2-naphthyl groups and such groups which are substituted by one or more of substituents D, defined above and examples of which have been given in relation to the same groups and atoms which may be represented by R^1 . The alkyl part may be substituted by one or more aryl groups, the maximum being dictated only by the number of substitutable positions and possibly also by steric constraints; however, from 1 to 3 aryl groups are normally preferred, 1 or 2 being more preferred and 1 being most preferred. Specific examples of the aralkyl amino groups include the benzylamino, N-(1-naphthylmethyl)amino, N-(2-naphthylmethyl)amino, phenethylamino, N-(α -methylbenzyl)amino, N-(3-phenylpropyl)amino, N-(2-phenylpropyl)amino, N-(1-phenylpropyl)amino, N-(4-phenylbutyl)amino, benzhydrylamino and tritylamino groups (of these, the benzylamino group is preferred), and such groups which are substituted by one or more of substituents D.

Where R^3 represents an alkanoylamino group, this may be a straight or branched chain group and has from 1 to 18 carbon atoms. Examples of such groups include

the formamido, acetamido, propionamido, butyramido, isobutyramido, valerylamino, isovalerylamino, pivaloylamino, hexanoylamino, heptanoylamino, octanoylamino, nonanoylamino, decanoylamino, lauroylamino, myristoylamino, palmitoylamino and stearoylamino groups, of which those groups having from 1 to 12 carbon atoms are preferred, those having from 2 to 10 carbon atoms are more preferred, and those having from 2 to 5 carbon atoms are most preferred, especially the acetamido, propionamido, butyramido, pivaloylamino, nonanoylamino and decanoylamino groups, of which the acetamido, propionamido, butyramido and pivaloylamino groups are most preferred.

Where R^3 represents an alkenoylamino group, this may be a straight or branched chain group and has from 3 to 6 carbon atoms. Examples of such groups include the acryloylamino, methacryloylamino, 2-butenoylamino, 2-pentenoylamino and 2-hexenoylamino groups, of which the acryloylamino and methacryloylamino groups are preferred.

Where R^3 represents a cycloalkylcarbonylamino group, this has from 4 to 8 carbon atoms, that is the cycloalkyl group itself has from 3 to 7 ring carbon atoms. Examples of such groups include the cyclopropylcarbonylamino, cyclobutylcarbonylamino, cyclopentylcarbonylamino, cyclohexylcarbonylamino and cycloheptylcarbonylamino groups, of which the cyclopropylcarbonylamino and cyclobutylcarbonylamino groups are particularly preferred.

Where R^3 represents arylcarbonylamino group, the aryl part is as defined above, and examples of such groups include the benzamido, 1-naphthoylamino, 2-naphthoylamino, o-, m- and p-toluoylamino, o-, m- and p-chlorobenzamido, o-, m- and p-fluorobenzamido, o-, m- and p-methoxybenzamido, 2,4-dichlorobenzamido, 2,4-difluorobenzamido and 2,4,6-trifluorobenzamido groups, preferably the benzamido group.

Where R^3 represents an alkoxy carbonylamino group, this may be a straight or branched chain alkoxy carbonylamino group having from 2 to 5 carbon atoms, that is the alkoxy part has from 1 to 4 carbon atoms, add examples include the methoxycarbonylamino, ethoxycarbonylamino, propoxycarbonylamino, isopropoxycarbonylamino, butoxycarbonylamino, isobutoxycarbonylamino, sec-butoxycarbonylamino and t-butoxycarbonylamino groups. Of these, we prefer those alkoxy carbonylamino groups having from 1 to 3 carbon atoms in the alkoxy part and the t-butoxycarbonylamino group, more preferably the methoxycarbonylamino, ethoxycarbonylamino and t-butoxycarbonylamino groups.

Where R^3 represents an aralkoxy carbonylamino group, the alkoxy part is an alkoxy group having from 1 to 4, preferably from 1 to 3, carbon atoms, such as those exemplified above in relation to R^1 , especially the methoxy, ethoxy, propoxy or isopropoxy groups. The aryl part is as defined above and has from 6 to 10, preferably 6 or 10, ring carbon atoms. Examples of such aryl groups include the phenyl, 1-naphthyl and 2-naphthyl groups and such groups which are substituted by one or more of substituents D, defined above and examples of which have been given in relation to the same groups and atoms which may be represented by R^1 . The alkoxy part may be substituted by one or more aryl groups, the maximum being dictated only by the number of substitutable positions and possibly also by steric constraints; however, from 1 to 3 aryl groups are normally pre-

ferred, 1 or 2 being more preferred and 1 being most preferred. Specific examples of the aralkyloxycarbonylamino groups include the benzyloxycarbonylamino, N-(1-naphthylmethoxycarbonyl)amino, N-(2-naphthylmethoxycarbonyl)amino, phenethyloxycarbonylamino, N-(α -methylbenzyloxycarbonyl)amino, N-(3-phenylpropoxycarbonyl)amino, N-(2-phenylpropoxycarbonyl)amino, N-(1-phenylpropoxycarbonyl)amino, N-(4-phenylbutoxycarbonyl)amino, benzhydryloxycarbonylamino and trityloxycarbonylamino groups (of these, the benzyloxycarbonylamino group is preferred), and such groups which are substituted by one or more of substituents D.

Y represents a group of formula —NH— or an oxygen or sulfur atom, preferably an oxygen or sulfur atom, and more preferably a sulfur atom.

R^3 may be at either the 2- or the 3- position of the tetrahydropyrrolopyridyl, tetrahydrothienopyridyl or tetrahydrofuropyridyl group, but is preferably at the 2-position, especially when the Y is an oxygen or sulfur atom, i.e. on the tetrahydrothienopyridyl or tetrahydrofuropyridyl group.

In the compounds of the present invention, the carbon atom to which the group represented by R^2 is attached is an assymetric carbon atom, and other carbon atoms may be assymetric, and the compounds accordingly form optical isomers. Although these are all represented herein by a single molecular formula, the present invention includes both the individual, isolated isomers and mixtures, including racemates thereof. Where stereospecific synthesis techniques are employed or optically active compounds are employed as starting materials, individual isomers may be prepared directly; on the other hand, if a mixture of isomers is prepared, the individual isomers may be obtained by conventional resolution techniques.

In addition, when the compounds of the present invention have one or more carbon-carbon double bonds or one or more disubstituted cycloalkyl moieties, they form cis and trans isomers. The present invention includes both the individual, isolated isomers and mixtures thereof.

The compounds of the present invention can form acid addition salts. There is no particular restriction on the nature of these salts, provided that, where they are intended for therapeutic use, they are pharmaceutically acceptable. Where they are intended for non-therapeutic uses, e.g. as intermediates in the preparation of other, and possibly more active, compounds, even this restriction does not apply. Examples of such acid addition salts include: salts with mineral acids, especially hydrohalic acids (such as hydrofluoric acid, hydrobromic acid, hydroiodic acid or hydrochloric acid), nitric acid, carbonic acid, sulfuric acid or phosphoric acid; salts with lower alkylsulfonic acids, such as methanesulfonic acid, trifluoromethanesulfonic acid or ethanesulfonic acid; salts with arylsulfonic acids, such as benzenesulfonic acid or p-toluenesulfonic acid; and salts with organic carboxylic acids, such as acetic acid, propionic acid, butyric acid, fumaric acid, tartaric acid, oxalic acid, malonic acid, maleic acid, malic acid, succinic acid, benzoic acid, mandelic acid, ascorbic acid, lactic acid, gluconic acid or citric acid.

The compounds of the present invention may also readily form hydrates and these, also, form part of the present invention.

Additionally, when R^3 represents an amino group or a substituted amino group, the resulting compound can

form a complex salt with a metal ion, and such complex salts also form part of the present invention. Examples of such complex salts include salts with calcium chloride, magnesium chloride, zinc chloride, ferric chloride, stannic chloride and nickel chloride.

Preferred classes of compounds of the present invention are those compounds of formulae (I) and tautomers and salts thereof in which:

(A) R^1 represents a hydrogen atom, an alkyl group having from 1 to 4 carbon atoms, a halogen atom, a fluoroalkyl group having from 1 to 4 carbon atoms and at least one fluorine atom, a hydroxy group, an alkoxy group having from 1 to 4 carbon atoms, a fluoroalkoxy group having from 1 to 4 carbon atoms and at least one fluorine atom, an alkylthio group having from 1 to 4 carbon atoms, a fluoroalkylthio group having from 1 to 4 carbon atoms and at least one fluorine atom, an amino group, an alkanoyl group having from 1 to 5 carbon atoms, a fluoroalkanoyl group having from 2 to 5 carbon atoms and at least one fluorine atom, an alkoxycarbonyl group having from 2 to 5 carbon atoms, a carbamoyl group, a cyano group, a nitro group, an alkanesulfonyl group having from 1 to 4 carbon atoms, a fluoroalkanesulfonyl group having from 1 to 4 carbon atoms and at least one fluorine atom, or a sulfamoyl group.

(B) R^2 represents an alkanoyl group having from 2 to 6 carbon atoms, a substituted alkanoyl group which has from 2 to 6 carbon atoms and which is substituted by at least one substituent selected from the group consisting of substituents A', defined below, a cycloalkylcarbonyl group having from 4 to 7 carbon atoms, a substituted cycloalkylcarbonyl group which has from 4 to 7 carbon atoms and which is substituted by at least one substituent selected from the group consisting of substituents A', defined below, a substituted benzoyl group having at least one fluorine substituent, or a 5,6-dihydro-1,4,2-dioxazin-3-yl group; and

said substituents A' are selected from the group consisting of fluorine atoms, chlorine atoms, hydroxy groups, methoxy groups, ethoxy groups and cyano groups.

(C) R^3 represents a hydrogen atom, a hydroxy group, an alkoxy group having from 1 to 4 carbon atoms, an alkoxymethoxy group in which the alkoxy part has from 1 to 4 carbon atoms, an alkanoyloxymethoxy group in which the alkanoyl part has from 1 to 5 carbon atoms, a benzyloxy group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D', defined below, an alkanoyloxy group having from 1 to 18 carbon atoms, an alkenoyloxy group having 3 or 4 carbon atoms, a cycloalkylcarbonyloxy group having from 4 to 7 carbon atoms, a benzoyloxy group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D', defined below, an alkoxycarbonyloxy group having from 2 to 5 carbon atoms, a benzyloxycarbonyloxy group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D', defined below, a phthalidyl group, a (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy group, a (5-phenyl-2-oxo-1,3-dioxolen-4-yl)methoxy group, a group of formula $\text{—NR}^a\text{R}^b$

wherein R^a and R^b are independently selected from the group consisting of hydrogen atoms, methyl and ethyl groups or R^a represents a hydrogen atom and R^b represents an alkanoyloxymethyl group in which the alkanoyl part has from 1 to 5

carbon atoms, a benzylamino group, an alkanoylamino group having from 1 to 18 carbon atoms, an alkenoylamino group having 3 or 4 carbon atoms, a cycloalkylcarbonylamino group having 6 or 7 carbon atoms, a benzoylamino group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D' , defined below, an alkoxycarbonylamino group having from 2 to 5 carbon atoms or a benzyloxycarbonylamino group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D' , defined below;

and

said substituents D' are selected from the group consisting of fluorine atoms, chlorine atoms, methyl groups and methoxy groups.

(D) Y represents an oxygen or sulfur atom.

Of these, we prefer those compounds in which R^1 is as defined in (A) above, R^2 is as defined in (B) above, R^3 is as defined in (C) above and Y is as defined in (D) above.

More preferred classes of compounds of the present invention are those compounds of formulae (I) and tautomers and salts thereof in which:

(E) R^1 represents a hydrogen atom, a methyl group, an ethyl group, a halogen atom, a methyl group substituted by at least one fluorine atom, a hydroxy group, a methoxy group, an ethoxy group, a methoxy group substituted by at least one fluorine atom, a methylthio group, a methylthio group substituted by at least one fluorine atom, a formyl group, an acetyl group, an acetyl group substituted by at least one fluorine atom, an alkoxycarbonyl group having from 2 to 4 carbon atoms, a carbamoyl group, a cyano group, a nitro group, a methanesulfonyl group, an ethanesulfonyl group, a methanesulfonyl group substituted by at least one fluorine atom, or a sulfamoyl group.

(F) R^2 represents an alkanoyl group having from 2 to 6 carbon atoms, a substituted alkanoyl group which has from 2 to 6 carbon atoms and which is substituted by at least one fluorine atom, a cycloalkylcarbonyl group having from 4 to 7 carbon atoms, or a substituted cycloalkylcarbonyl group which is substituted by at least one fluorine atom.

(G) R^3 represents a hydrogen atom, a hydroxy group, a methoxy group, an ethoxy group, a t-butoxy group, a methoxymethoxy group, an alkanoyloxymethoxy group in which the alkanoyl part has from 1 to 5 carbon atoms, a benzyloxy group, an alkanoyloxy group having from 1 to 12 carbon atoms, an alkenoyloxy group having 3 or 4 carbon atoms, a cycloalkylcarbonyloxy group having from 4 to 7 carbon atoms, a benzoyloxy group, an alkoxycarbonyloxy group having from 2 to 5 carbon atoms, a benzyloxycarbonyloxy group, a phthalidyloxy group, a (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy group, a (5-phenyl-2-oxo-1,3-dioxolen-4-yl)methoxy group, an amino group or a t-butoxycarbonylamino group.

Of these, we prefer those compounds in which R^1 is as defined in (E) above, R^2 is as defined in (F) above, R^3 is as defined in (G) above and Y is as defined in (D) above.

Still more preferred classes of compounds of the present invention are those compounds of formulae (I) and tautomers and salts thereof in which:

(H) R^1 represents a halogen atom, a trifluoromethyl group, a hydroxy group, a difluoromethoxy group, a trifluoromethoxy group, a difluoromethylthio group, a trifluoromethylthio group, a formyl group, an acetyl group, a trifluoroacetyl group, a cyano group or a nitro group.

(I) R^3 represents a hydrogen atom, a hydroxy group, a pivaloyloxymethoxy group, an alkanoyloxy group having from 2 to 10 carbon atoms, an alkoxycarbonyloxy group having from 2 to 5 carbon atoms or a (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy group.

(J) Y represents a sulfur atom.

Of these, we prefer those compounds in which R^1 is as defined in (H) above, R^2 is as defined in (F) above, R^3 is as defined in (I) above and Y is as defined in (J) above.

The most preferred classes of compounds of the present invention are those compounds of formulae (I) and tautomers and salts thereof in which:

(K) R^1 represents a fluorine or chlorine atom.

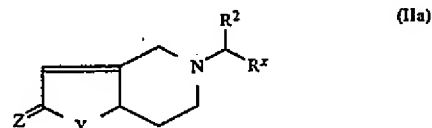
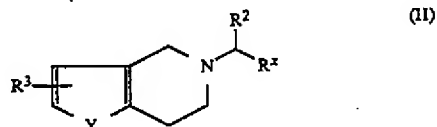
(L) R^2 represents an acetyl group, a propionyl group, a substituted acetyl or propionyl group which is substituted by at least one fluorine atom, a cyclopropylcarbonyl group, cyclobutylcarbonyl group, or a substituted cyclopropylcarbonyl or cyclobutylcarbonyl group which is substituted by at least one fluorine atom.

(M) R^3 represents a hydrogen atom, a hydroxy group, a pivaloyloxymethoxy group, an alkanoyloxy group having from 2 to 6 carbon atoms or an alkoxycarbonyloxy group having from 2 to 5 carbon atoms.

Of these, we prefer those compounds in which R^1 is as defined in (K) above, R^2 is as defined in (L) above, R^3 is as defined in (M) above and Y is as defined in (J) above.

In all of the above classes of compounds, we prefer that n should be from 1 to 3, especially 1 or 2, and most preferably 1.

Specific examples of preferred compounds of the present invention are those compounds of formula (II) or (IIa), in which R^x , R^2 , R^3/Z and Y are as defined in the following Table 1. In the column headed " R^3/Z ", the " R^3 " applies to compounds of formula (II), whilst " Z " applies to compounds of formula (IIa):



In the Table, the following abbreviations are used to refer to certain substituent groups:

Ac	acetyl
Acr	acryloyl
tBoc	t-butoxycarbonyl
Boz	benzoyl
cBu	cyclobutyl
tBu	t-butyl
Bun	butenyl
Byr	butyryl
iByr	isobutyryl
Bz	benzyl
Bzc	benzyloxycarbonyl
Car	carbamoyl
Dcn	decanoyl
Ddoz	3,6-dihydro-1,4,2-dioxazin-3-yl
Et	ethyl
Etc	ethoxycarbonyl
Fo	formyl
cHp	cycloheptyl
cHx	cyclohexyl
Hxn	hexanoyl
Lau	lauroyl
Me	methyl
Mec	methoxycarbonyl
Mod	(5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl
Nnn	nonanoyl
Plt	palmitoyl
Ph	phenyl
Phth	phthalidyl
Piv	pivaloyl
cPn	cyclopentyl
cPr	cyclopropyl
Prn	propionyl
Va	valeryl
iVa	isovaleryl

TABLE 1-continued

Cpd. No.	For- mula	R ¹	R ²	R ³ /Z	Y
37	(II)	2-MeSO ₂ Ph	Prn	H	S
38	(II)	2-EtSO ₂ Ph	Prn	H	S
39	(II)	2-CF ₃ SO ₂ Ph	Prn	H	S
40	(II)	4-CarPh	Prn	H	S
41	(II)	3-NH ₂ SO ₂ Ph	Prn	H	S
42	(II)	2-FoPh	Prn	H	S
43	(II)	2-AcPh	Prn	H	S
44	(II)	2-CF ₃ COPh	Prn	H	S
45	(II)	2,6-diFPh	Prn	H	S
46	(II)	2-F, 6-ClPh	Prn	H	S
47	(II)	2,4,6-triFPh	Prn	H	S
48	(II)	2,3,4,5,6-pentaFPh	Prn	H	S
49	(II)	2-F, 6-CNPh	Prn	H	S
50	(II)	2-F, 6-NO ₂ Ph	Prn	H	S
51	(II)	2,6-diF, 4-MePh	Prn	H	S
52	(II)	2,4-diClPh	Prn	H	S
53	(II)	2-F, 4-HOPh	Prn	H	S
54	(II)	2-Cl, 4-MecPh	Prn	H	S
55	(II)	2-F, 6-CHF ₂ OPh	Prn	H	S
56	(II)	2-Cl, 4-EtPh	Prn	H	S
57	(II)	2-F, 5-EtOPh	Prn	H	S
58	(II)	Ph	cPrCO	H	S
59	(II)	2-FPh	cPrCO	H	S
60	(II)	2-ClPh	cPrCO	H	S
61	(II)	2-BrPh	cPrCO	H	S
62	(II)	2-IPh	cPrCO	H	S
63	(II)	2-HOPh	cPrCO	H	S
64	(II)	2-NO ₂ Ph	cPrCO	H	S
65	(II)	2-Cl, 5-NH ₂ Ph	cPrCO	H	S
66	(II)	2-CNPh	cPrCO	H	S
67	(II)	2-F, 5-HOOCPh	cPrCO	H	S
68	(II)	2-F, 4-MePh	cPrCO	H	S
69	(II)	2-CF ₃ Ph	cPrCO	H	S
70	(II)	2-F, 4-MeOPh	cPrCO	H	S
71	(II)	2-CHF ₂ OPh	cPrCO	H	S
72	(II)	2-CF ₃ OPh	cPrCO	H	S
73	(II)	3-CH ₂ FOPh	cPrCO	H	S
74	(II)	4-MeSPh	cPrCO	H	S
75	(II)	2-CHF ₂ SPh	cPrCO	H	S
76	(II)	3-CF ₃ SPh	cPrCO	H	S
77	(II)	2-MeSO ₂ Ph	cPrCO	H	S
78	(II)	2-EtSO ₂ Ph	cPrCO	H	S
79	(II)	2-CF ₃ SO ₂ Ph	cPrCO	H	S
80	(II)	4-CarPh	cPrCO	H	S
81	(II)	3-NH ₂ SO ₂ Ph	cPrCO	H	S
82	(II)	2-FoPh	cPrCO	H	S
83	(II)	2-AcPh	cPrCO	H	S
84	(II)	2-CF ₃ COPh	cPrCO	H	S
85	(II)	2,6-diFPh	cPrCO	H	S
86	(II)	2-F, 6-ClPh	cPrCO	H	S
87	(II)	2,4,6-triFPh	cPrCO	H	S
88	(II)	2,3,4,5,6-pentaFPh	cPrCO	H	S
89	(II)	2-F, 6-CNPh	cPrCO	H	S
90	(II)	2-F, 6-NO ₂ Ph	cPrCO	H	S
91	(II)	2,6-diF, 4-MePh	cPrCO	H	S
92	(II)	2,4-diClPh	cPrCO	H	S
93	(II)	2-F, 4-HOPh	cPrCO	H	S
94	(II)	2-Cl, 4-EtPh	cPrCO	H	S
95	(II)	2-F, 6-CHF ₂ OPh	cPrCO	H	S
96	(II)	2-Cl, 4-EtPh	cPrCO	H	S
97	(II)	2-F, 5-EtOPh	cPrCO	H	S
98	(II)	2-FPh	3-FPrn	H	S
99	(II)	2-ClPh	3-FPrn	H	S
100	(II)	2-CNPh	3-FPrn	H	S
101	(II)	2,6-diFPh	3-FPrn	H	S
102	(II)	2-F, 6-ClPh	3-FPrn	H	S
103	(II)	2-F, 6-CNPh	3-FPrn	H	S
104	(II)	2-NO ₂ Ph	3-FPrn	H	S
105	(II)	2-F, 4-CNPh	3-FPrn	H	S
106	(II)	2-FPh	cBuCO	H	S

TABLE 1

Cpd. No.	For- mula	R ¹	R ²	R ³ /Z	Y
1	(II)	Ph	Ddoz	H	S
2	(II)	2-FPh	Ddoz	H	S
3	(II)	2-ClPh	Ddoz	H	S
4	(II)	2-CNPh	Ddoz	H	S
5	(II)	2-NO ₂ Ph	Ddoz	H	S
6	(II)	2-CHF ₂ Ph	Ddoz	H	S
7	(II)	2,6-diFPh	Ddoz	H	S
8	(II)	2-F, 6-ClPh	Ddoz	H	S
9	(II)	2-FPh	Ac	H	S
10	(II)	2-ClPh	Ac	H	S
11	(II)	2-CNPh	Ac	H	S
12	(II)	2-NO ₂ Ph	Ac	H	S
13	(II)	2-CF ₃ Ph	Ac	H	S
14	(II)	2,6-diFPh	Ac	H	S
15	(II)	2-F, 6-ClPh	Ac	H	S
16	(II)	2,4-diFPh	Ac	H	S
17	(II)	2-F, 6-CNPh	Ac	H	S
18	(II)	Ph	Prn	H	S
19	(II)	2-FPh	Prn	H	S
20	(II)	2-ClPh	Prn	H	S
21	(II)	2-BrPh	Prn	H	S
22	(II)	2-IPh	Prn	H	S
23	(II)	2-HOPh	Prn	H	S
24	(II)	2-NO ₂ Ph	Prn	H	S
25	(II)	2-Cl, 5-NH ₂ Ph	Prn	H	S
26	(II)	2-CNPh	Prn	H	S
27	(II)	2-F, 5-HOOCPh	Prn	H	S
28	(II)	2-F, 4-MePh	Prn	H	S
29	(II)	2-CF ₃ Ph	Prn	H	S
30	(II)	2-F, 4-MeOPh	Prn	H	S
31	(II)	2-CHF ₂ OPh	Prn	H	S
32	(II)	2-CF ₃ OPh	Prn	H	S
33	(II)	3-CH ₂ FOPh	Prn	H	S
34	(II)	4-MeSPh	Prn	H	S
35	(II)	2-CHF ₂ SPh	Prn	H	S
36	(II)	3-CF ₃ SPh	Prn	H	S

TABLE 1-continued

Cpd. No.	Formula	R ¹	R ²	R ³ /Z	Y
107	(II)	2-ClPh	cBuCO	H	S
108	(II)	2-CNPh	cBuCO	H	S
109	(II)	2-FPh	HOCH ₂ CO	H	S
110	(II)	2-ClPh	HOCH ₂ CO	H	S
111	(II)	2-CNPh	CF ₃ CO	H	S
112	(II)	2-FPh	CF ₃ CO	H	S
113	(II)	2-ClPh	CF ₃ CO	H	S
114	(II)	2-FPh	Fo	H	S
115	(II)	2-ClPh	Fo	H	S
116	(II)	2-FPh	Byr	H	S
117	(II)	2-ClPh	Byr	H	S
118	(II)	2-FPh	iByr	H	S
119	(II)	2-ClPh	iByr	H	S
120	(II)	2-FPh	Va	H	S
121	(II)	2-ClPh	Va	H	S
122	(II)	2-FPh	Piv	H	S
123	(II)	2-F, 6-ClPh	Piv	H	S
124	(II)	2-FPh	iVa	H	S
125	(II)	2-ClPh	Han	H	S
126	(II)	2-FPh	Dcn	H	S
127	(II)	2-ClPh	1-Bun	H	S
128	(II)	2-FPh	cPhCO	H	S
129	(II)	2-FPh	cHxCO	H	S
130	(II)	2-FPh	cHpCO	H	S
131	(II)	2-FPh	CH ₂ FCO	H	S
132	(II)	2-FPh	CHF ₂ CO	H	S
133	(II)	2-ClPh	CHF ₂ CO	H	S
134	(II)	2-CNPh	CHF ₂ CO	H	S
135	(II)	2-FPh	MeO—CH ₂ CO	H	S
136	(II)	2-ClPh	MeO—CH ₂ CO	H	S
137	(II)	2-FPh	NC—CH ₂ CO	H	S
138	(II)	2-ClPh	NC—CH ₂ CO	H	S
139	(II)	2,6-diFPh	NC—CH ₂ CO	H	S
140	(II)	2-FPh	3-ClPm	H	S
141	(II)	2-ClPh	3-ClPm	H	S
142	(II)	2-FPh	3-HOPm	H	S
143	(II)	2-ClPh	3-HOPm	H	S
144	(II)	2-FPh	3-MeOPm	H	S
145	(II)	2-FPh	3-CNPrn	H	S
146	(II)	2-FPh	4-FByr	H	S
147	(II)	2-ClPh	4-ClByr	H	S
148	(II)	2-FPh	4-FBoz	H	S
149	(II)	2-ClPh	4-FBoz	H	S
150	(II)	2-CNPh	4-FBoz	H	S
151	(II)	2-FPh	2,4-diFBoz	H	S
152	(II)	2-ClPh	2,4-diFBoz	H	S
153	(II)	2-NO ₂ Ph	2,4-diFBoz	H	S
154	(II)	2-FPh	3-BrPm	H	S
155	(II)	2-FPh	3-IPm	H	S
156	(II)	2-FPh	Ac	H	O
157	(II)	2-ClPh	Ac	H	O
158	(II)	2-CNPh	Ac	H	O
159	(II)	2-NO ₂ Ph	Ac	H	O
160	(II)	2-FPh	Pm	H	O
161	(II)	2-ClPh	Pm	H	O
162	(II)	2-CNPh	Pm	H	O
163	(II)	2-NO ₂ Ph	Pm	H	O
164	(II)	2-FPh	3-FPm	H	O
165	(II)	2-ClPh	3-FPm	H	O
166	(II)	2-CNPh	3-FPm	H	O
167	(II)	2-NO ₂ Ph	3-FPm	H	O
168	(II)	2-FPh	cPrCO	H	O
169	(II)	2-ClPh	cPrCO	H	O
170	(II)	2-CNPh	cPrCO	H	O
171	(II)	2-NO ₂ Ph	cPrCO	H	O
172	(II)	2,6-diFPh	cPrCO	H	O
173	(II)	2-F, 6-ClPh	cPrCO	H	O
174	(II)	2,6-diFPh	4-FBoz	H	S
175	(II)	2-FPh	cPrCO	2-NO ₂	S
176	(II)	2-FPh	cPrCO	2-NH ₂	O
177	(II)	2-FPh	cPrCO	2-NH ₂	S
178	(II)	2-FPh	cPrCO	2-AcNH	O
179	(II)	2-FPh	cPrCO	2-AcNH	S
180	(II)	2-FPh	cPrCO	2-ByrNH	O
181	(II)	2-FPh	cPrCO	2-ByrNH	S
182	(II)	2-FPh	cPrCO	2-PivNH	S
183	(II)	2-FPh	cPrCO	2-tBocNH	O
184	(II)	2-FPh	cPrCO	2-tBocNH	S
185	(II)	2-FPh	cPrCO	2-HO	O
186	(II)	2-ClPh	cPrCO	2-HO	S

TABLE 1-continued

Cpd. No.	Formula	R ¹	R ²	R ³ /Z	Y
187	(II)	2-FPh	Pm	2-HO	S
188	(II)	2-FPh	cPrCO	2-HO	S
189	(II)	2-FPh	cPrCO	2-AcO	O
190	(II)	2-FPh	cPrCO	2-AcO	S
191	(II)	2-FPh	cPrCO	2-PmO	O
192	(II)	2-FPh	cPrCO	2-PmO	S
193	(II)	2-FPh	cPrCO	2-ByrO	O
194	(II)	2-FPh	cPrCO	2-ByrO	S
195	(II)	2-FPh	cPrCO	2-PivO	O
196	(II)	2-FPh	cPrCO	2-PivO	S
197	(II)	2-FPh	cPrCO	2-VaO	S
198	(II)	2-FPh	cPrCO	2-HxnO	S
199	(II)	2-FPh	cPrCO	2-NanO	S
200	(II)	2-FPh	cPrCO	2-DcnO	S
201	(II)	2-FPh	cPrCO	2-PhO	S
202	(II)	2-FPh	cPrCO	2-BozO	S
203	(II)	2-FPh	cPrCO	2-tBocO	S
204	(II)	2-FPh	cPrCO	2-tBuO	S
205	(II)	2-FPh	cPrCO	2-BzO	S
206	(II)	2-FPh	cPrCO	2-MeOCH ₂ O	S
207	(II)	2-FPh	cPrCO	2-PivOCH ₂ O	S
208	(II)	2-FPh	cPrCO	2-PhuO	S
209	(II)	2-FPh	cPrCO	2-ModO	S
210	(II)	2-FPh	cPrCO	2-MeO	S
211	(II)	2-FPh	cPrCO	2-EtO	S
212	(II)	2-FPh	cPrCO	2-LauO	S
213	(II)	2-FPh	cPrCO	2-AcrO	S
214	(II)	2-FPh	cPrCO	2-cHxCOO	S
215	(II)	2-FPh	cPrCO	2-MecO	S
216	(II)	2-FPh	cPrCO	2-EtO	S
217	(II)	2-FPh	cPrCO	2-FoNH	S
218	(II)	2-FPh	cPrCO	2-PmNH	S
219	(II)	2-FPh	cPrCO	2-MeNH	S
220	(II)	2-FPh	cPrCO	2-EtNH	S
221	(II)	2-FPh	cPrCO	2-NMe ₂	S
222	(II)	2-FPh	cPrCO	2-AcrNH	S
223	(II)	2-FPh	cPrCO	2-cHxCONH	S
224	(II)	2-FPh	cPrCO	2-MecNH	S
225	(II)	2-FPh	cPrCO	2-EtNH	S
226	(II)	2-FPh	cPrCO	2-BozNH	S
227	(II)	2-FPh	cPrCO	2-BozO	O
228	(II)	2-FPh	cPrCO	2-tBocO	O
229	(II)	2-FPh	Pm	2-NO ₂	S
230	(II)	2-FPh	cPrCO	2-BzO	S
231	(II)	2-FPh	cPrCO	2-BzNH	S
232	(IIa)	2-FPh	cPrCO	O	O
233	(IIa)	2-ClPh	cPrCO	O	O
234	(IIa)	2-FPh	Pm	O	S
235	(IIa)	2-FPh	cPrCO	O	S
236	(II)	2-FPh	Pm	2-AcO	S
237	(II)	2-FPh	Pm	2-PmO	S
238	(II)	2-FPh	Pm	2-ByrO	S
239	(II)	2-FPh	Pm	2-PivO	S
240	(II)	2-FPh	Pm	2-VaO	S
241	(II)	2-FPh	Pm	2-HxnO	S
242	(II)	2-FPh	Pm	2-MecO	S
243	(II)	2-FPh	Pm	2-EtO	S
244	(II)	2-FPh	Pm	2-tBocO	S
245	(II)	2-FPh	Pm	2-BozO	S
246	(II)	2-FPh	Pm	2-NH ₂	S
247	(II)	2-FPh	Pm	2-AcNH	S
248	(II)	2-FPh	Pm	2-PmNH	S
249	(II)	2-FPh	Pm	2-ByrNH	S
250	(II)	2-FPh	Pm	2-tBocNH	S
251	(II)	2-FPh	Pm	2-BzNH	S
252	(II)	2-ClPh	cPrCO	2-AcO	S
253	(II)	2-ClPh	cPrCO	2-PmO	S
254	(II)	2-ClPh	cPrCO	2-ByrO	S
255	(II)	2-ClPh	cPrCO	2-PivO	S
256	(II)	2-ClPh	cPrCO	2-VaO	S
257	(II)	2-ClPh	cPrCO	2-HxnO	S
258	(II)	2-ClPh	cPrCO	2-MecO	S
259	(II)	2-ClPh	cPrCO	2-EtO	S
260	(II)	2-ClPh	cPrCO	2-tBocO	S
261	(II)	2-ClPh	cPrCO	2-BozO	S
262	(II)	2-ClPh	cPrCO	2-NH ₂	S
263	(II)	2-ClPh	cPrCO	2-AcNH	S
264	(II)	2-ClPh	cPrCO	2-PmNH	S
265	(II)	2-ClPh	cPrCO	2-ByrNH	S
266	(II)	2-ClPh	cPrCO	2-tBocNH	S

TABLE 1-continued

Cpd. No.	For- mula	R ¹	R ²	R ³ /Z	Y
267	(II)	2-ClPh	cPrCO	2-BzNH	S
268	(II)	2-FPh	cPrCO	2-MeOCH ₂ NH	S
269	(II)	2-FPh	cPrCO	2-PhthNH	S
270	(II)	2-FPh	cPrCO	2-ModNH	S
271	(II)	2-FPh	cPrCO	2-PivOCH ₂ NH	S
272	(II)	2-FPh	2-FcPrCO	H	S
273	(II)	2-FPh	2-FcPrCO	H	O
274	(II)	2-FPh	2-FcPrCO	2-OH	S
275	(IIa)	2-FPh	2-FcPrCO	O	S
276	(II)	2-FPh	2-FcPrCO	2-AcO	S
277	(II)	2-FPh	2-FcPrCO	2-ByrO	S
278	(II)	2-FPh	2-FcPrCO	2-PivO	S
279	(II)	2-FPh	2-FcPrCO	2-PivOCH ₂ O	S
280	(II)	2-ClPh	2-FcPrCO	H	S
281	(II)	2-ClPh	2-FcPrCO	2-OH	S
282	(IIa)	2-ClPh	2-FcPrCO	O	S
283	(II)	2-ClPh	2-FcPrCO	2-AcO	S
284	(II)	2-ClPh	2-FcPrCO	2-ByrO	S
285	(II)	2-ClPh	2-FcPrCO	2-PivO	S
286	(II)	2-ClPh	2-FcPrCO	2-PivOCH ₂ O	S
287	(II)	2-FPh	2,2-diFcPrCO	H	S
288	(II)	2-FPh	2,2-diFcPrCO	2-OH	S
289	(IIa)	2-FPh	2,2-diFcPrCO	O	S
290	(II)	2-FPh	2,2-diFcPrCO	2-AcO	S
291	(II)	2-FPh	2,2-diFcPrCO	2-ByrO	S

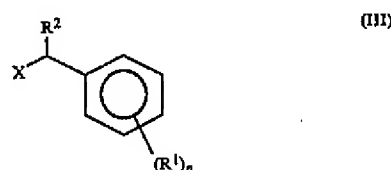
Of the compounds listed above, the following are preferred, that is to say Compounds No. 2, 3, 7, 9, 10, 11, 12, 19, 20, 24, 26, 29, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 89, 90, 98, 99, 106, 107, 108, 109, 111, 112, 113, 114, 116, 117, 118, 119, 120, 121, 122, 124, 125, 128, 129, 131, 132, 133, 135, 137, 140, 142, 144, 149, 151, 156, 160, 168, 177, 184, 186, 187, 188, 190, 192, 194, 196, 197, 198, 199, 200, 201, 203, 204, 206, 207, 208, 209, 210, 233, 234, 235, 236, 238, 239, 252, 253, 254, 255, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289 and 290, of which Compounds No. 9, 10, 19, 20, 59, 60, 63, 64, 66, 69, 71, 72, 75, 76, 83, 84, 85, 86, 98, 106, 113, 116, 118, 120, 122, 125, 128, 129, 131, 132, 186, 187, 188, 40 190, 192, 194, 196, 197, 198, 199, 200, 203, 207, 209, 233, 234, 235, 236, 238, 239, 252, 253, 254, 255, 274, 275, 276, 277, 278, 279, 281, 282, 283, 284, 285 and 286 are more preferred.

The most preferred compounds are Compounds No.:

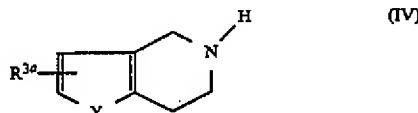
19. 5-(2-Fluoro- α -propionylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
59. 5-(α -Cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
60. 5-(2-Chloro-60 -cyclopropylcarbonylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
190. 2-Acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
192. 5-(60 Cyclopropylcarbonyl-2-fluorobenzyl)-2-propionyloxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
194. 2-Butyryloxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
196. 5-(α -Cyclopropylcarbonyl-2-fluorobenzyl)-2-pivaloyloxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
197. 5-(α -Cyclopropylcarbonyl-2-fluorobenzyl)-2-valeryloxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;

198. 5-(α -Cyclopropylcarbonyl-2-fluorobenzyl)-2-hexanoyloxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
203. 2-t-Butoxycarbonyloxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
207. 5-(α -Cyclopropylcarbonyl-2-fluorobenzyl)-2-pivaloyloxymethoxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
233. 5-(2-Chloro- α -cyclopropylcarbonylbenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine and its tautomer;
234. 5-(2-Fluoro- α -propionylbenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine and its tautomer;
235. 5-(α -Cyclopropylcarbonyl-2-fluorobenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine and its tautomer;
252. 2-Acetoxy-5-(2-chloro- α -cyclopropylcarbonylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
275. 5-[α -(2-Fluorocyclopropylcarbonyl-2-fluorobenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine and its tautomer;
276. 2-Acetoxy-5-[α -(2-fluorocyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine.

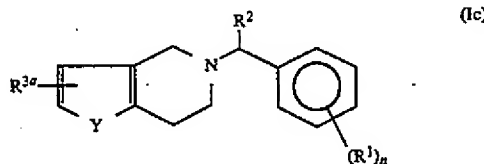
The compounds of the present invention can be prepared by a variety of methods, whose general techniques are known in the art for the preparation of compounds of this type. For example, they may be prepared by reacting a compound of formula (III):



(in which R¹, R² and n are as defined above and X represents a halogen atom, for example a fluorine, chlorine, bromine or iodine atom, preferably a chlorine or bromine atom) with a compound of formula (IV):



(in which Y is as defined above and R^{3a} represents a hydrogen atom or a hydroxy or nitro group) to give a compound of formula (Ic):

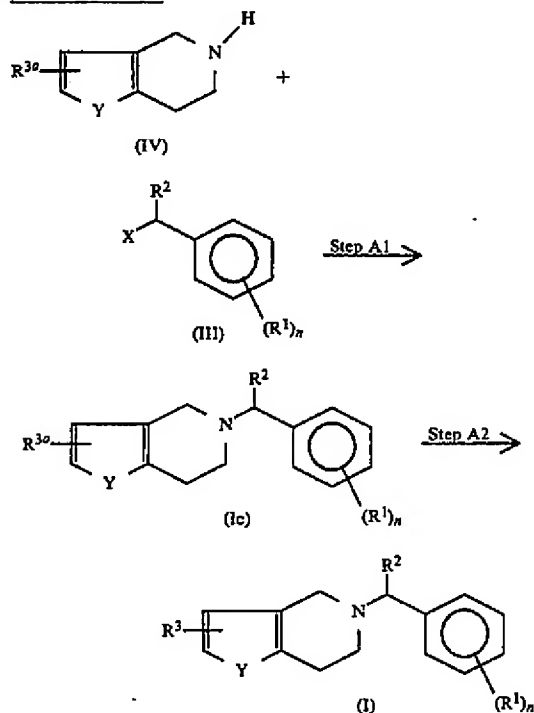


(in which R¹, R², R^{3a}, n and Y are as defined above). If required, this compound of formula (Ic) may then be subjected to one or more appropriate reactions, as explained in more detail hereafter, to convert the hy-

droxy or nitro group represented by R^{3a} to any other group represented by R^3 , as defined above.

These reactions may be summarized in the following Reaction Scheme A:

Reaction Scheme A:



In the above formulae, R^1 , R^2 , R^3 , R^{3a} , X , Y and n are as defined above.

In Step A1 of this Reaction Scheme, the substituted benzyl halide of formula (III) is reacted with a condensed hydronaphthyl compound of formula (IV), to give the compound of formula (Ic). This reaction may be carried out in the presence or absence of an inert solvent (preferably in the presence of an inert solvent) and in the presence or absence of a base (preferably in the presence of a base).

There is no specific limitation on the nature of the base employed, and any base known for use in reactions of this type may equally be used here. Examples of suitable bases include: organic amines, such as triethylamine, tributylamine, N-methylmorpholine, pyridine, 4-dimethylaminopyridine, picoline, lutidine, collidine, 1,8-diazabicyclo[5.4.0]undec-7-ene or 1,5-diazabicyclo[4.3.0]non-5-ene; alkali metal alkoxides, such as sodium methoxide, sodium ethoxide or potassium t-butoxide; alkali metal carbonates, such as sodium carbonate or potassium carbonate; and alkali metal hydroxides, such as sodium hydroxide or potassium hydroxide. Of these, the alkali metal carbonates are preferred. The amount of base employed is not critical, but we would generally recommend an amount of base of from an equimolar amount to 5 times the equimolar amount with respect to the starting material of formula (III). Where an excess of the starting material of formula (IV) is employed, this may also function as the base. Also, if an excess of an organic amine is employed as the base, this may additionally serve as the solvent.

The reaction is normally and preferably effected in the presence of a solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or on the reagents involved and that it can dissolve the reagents, at least to some extent. Examples of suitable solvents include: ethers, such as diethyl ether, tetrahydrofuran or dioxane; ketones, such as acetone or methyl ethyl ketone; esters, such as ethyl acetate; alcohols, such as methanol, ethanol, propanol, isopropanol or butanol; nitriles, such as acetonitrile; amides, such as N,N-dimethylformamide, N,N-dimethyl acetamide, N-methyl-2-pyrrolidone or hexamethyl phosphoric triamide; and sulfoxides, such as dimethyl sulfoxide. Of these, the amides or the sulfoxides are preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. In general, we find it convenient to carry out the reaction at a temperature of from 0° C. to 200° C. (more preferably at from about room temperature to 150° C.). The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the reagents and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from 1 to 24 hours (more preferably from 2 to 15 hours) will usually suffice.

After completion of the reaction, the desired compound of formula (Ic) can be obtained from the reaction mixture by conventional means. For example, if the compound is produced immediately in the form of crystals, these can be separated simply by filtration. Alternatively, a suitable recovery procedure comprises: adding water; neutralizing the mixture, if necessary; extracting the mixture with a water-immiscible organic solvent; drying the extract; and distilling the solvent off. If necessary, the product thus obtained can be further purified by conventional means, such as recrystallization or the various chromatography techniques, for example preparative thin layer chromatography or column chromatography, notably column chromatography.

In the optional second step of this reaction, Step A2, the resulting compound of formula (Ic) is converted, if desired, to a compound of formula (I). This reaction may involve any one or more of the following reactions:

- (1) Where R^{3a} represents a hydroxy group, alkylation, aralkylation or acylation of this hydroxy group;
- (2) Where R^{3a} represents a nitro group, conversion of this nitro group to an amino group;
- (3) Alkylation, aralkylation or acylation of the amino group obtained as described in (2) above.

Alkylation, aralkylation or acylation of the hydroxy group in Step A2(1) is carried out in an inert solvent and in the presence of a base by reacting a hydroxy compound of formula (Ic) (R^{3a} represents a hydroxy group) with a corresponding alkylating, aralkylating or acylating agent, for example an alkyl halide, aralkyl halide, acyl halide or acid anhydride. The nature of this compound will, of course, depend on the nature of the group which it is desired to introduce into the compound of formula (I). However, examples of suitable compounds are as follows:

alkyl halides having from 1 to 4 carbon atoms, such as methyl iodide, ethyl bromide, ethyl iodide, propyl chloride, propyl bromide, butyl chloride or butyl iodide;

aralkyl halides having from 7 to 14 carbon atoms, such as benzyl chloride, benzyl bromide, p-methylbenzyl chloride, p-methoxybenzyl chloride, p-chlorobenzyl chloride, p-fluorobenzyl chloride or naphthylmethyl chloride;

alkyl halides from 1 to 4 carbon atoms which are substituted by an alkoxy group having from 1 to 4 carbon atoms, by an alkanoyloxy group having from 1 to 6 carbon atoms or by an arylcarbonyloxy group having from 7 to 11 carbon atoms, such as methoxy methyl chloride, 1-methoxyethyl chloride, 2-methoxyethyl chloride, 1-methoxypropyl chloride, 1-methoxybutyl chloride, ethoxymethyl chloride, propoxymethyl chloride, butoxymethyl chloride, acetoxymethyl chloride, 1-acetoxyethyl chloride, 2-acetoxyethyl chloride, 1-acetoxypropyl chloride, 1-acetoxybutyl chloride, propionyloxymethyl chloride, butyryloxymethyl chloride, valeryloxymethyl chloride, pivaloyloxymethyl chloride, benzoyloxymethyl chloride, 1-benzoyloxyethyl chloride, p-methylbenzoyloxymethyl chloride, p-methoxybenzoyloxymethyl chloride, p-chlorobenzoyloxymethyl chloride, p-fluorobenzoyloxymethyl chloride or naphthoyloxymethyl chloride;

alkanoyl halides having from 2 to 18 carbon atoms or a mixed acid anhydride of one such corresponding acid with formic acid, such as acetyl chloride, propionyl chloride, butyryl chloride, butyryl bromide, valeryl chloride, isovaleryl chloride, pivaloyl chloride, hexanoyl chloride, nonanoyl chloride, decanoyl chloride, lauroyl chloride, palmitoyl chloride, stearoyl chloride, mixed acid anhydride of formic acid and acetic acid, acetic anhydride, propionic anhydride or butyric anhydride;

alkenoyl chlorides having from 3 to 6 carbon atoms, such as acryloyl chloride, methacryloyl chloride, crotonoyl chloride or 2-hexenoyl chloride;

cycloalkanecarbonyl halides having from 3 to 7 carbon atoms in the cycloalkane part, such as cyclopropanecarbonyl chloride, cyclobutanecarbonyl chloride, cyclopentanecarbonyl chloride, cyclohexanecarbonyl chloride or cycloheptanecarbonyl chloride;

arylcarbonyl halides having from 6 to 10 carbon atoms in the aryl part, such as benzoyl chloride, p-methylbenzoyl chloride, p-methoxybenzoyl chloride, p-chlorobenzoyl chloride, p-fluorobenzoyl chloride or naphthoyl chloride;

alkoxycarbonyl halides having from 1 to 4 carbon atoms in the alkoxy part, or an alkyl carbonate anhydride having from 1 to 4 carbon atoms in the alkyl part, such as methoxycarbonyl chloride, ethoxycarbonyl chloride, propoxycarbonyl chloride, isopropoxycarbonyl chloride, butoxycarbonyl chloride, t-butoxycarbonyl chloride, dimethyl dicarbonate, diethyl dicarbonate, dipropyl dicarbonate, diisopropyl dicarbonate, dibutyl dicarbonate or di-t-butyl dicarbonate;

aralkyloxycarbonyl halides having from 7 to 14 carbon atoms in the aralkyl part, such as benzyl oxycarbonyl chloride, p-methylbenzyloxycarbonyl chloride, p-methoxybenzyloxycarbonyl chloride, p-chlorobenzoyloxycarbonyl chloride, p-fluorobenzoyloxycarbonyl chloride or naphthylmethoxycarbonyl chloride;

phthalidyl halides, such as phthalidyl chloride; or substituted methyl halides, such as (5-methyl- or 5-phenyl-2-oxo-1,3-dioxolen-4-yl)methyl chloride.

The base employed is not critical to the invention, provided that it has no adverse effect on other parts of the molecule, and any base commonly used in reactions of this type may equally be used here. Examples of suitable bases include: alkali metal hydrides, such as lithium hydride or sodium hydride; alkali metal alkoxides, such as sodium methoxide, sodium ethoxide or potassium t-butoxide; alkali metal carbonates, such as sodium carbonate or potassium carbonate; and alkali metal hydroxides, such as sodium hydroxide or potassium hydroxide. Of these, the alkali metal hydrides are preferred.

The reaction is normally and preferably effected in the presence of a solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or on the reagents involved and that it can dissolve the reagents, at least to some extent. Examples of suitable solvents include: ethers, such as diethyl ether, tetrahydrofuran or dioxane; ketones, such as acetone or methyl ethyl ketone; esters, such as ethyl acetate; nitriles, such as acetonitrile; amides, such as N,N-dimethylformamide, N,N-dimethylacetamide, N-methyl-2-pyrrolidone or hexamethylphosphoric triamide; and sulfoxides, such as dimethyl sulfoxide. Of these, the amides are preferred.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. In general, we find it convenient to carry out the reaction at a temperature of from -10°C. to 100°C. (more preferably from 0°C. to 50°C.), although this may vary, depending on the nature of the compound of formula (Ic) and the solvent. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the reagents and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from 30 minutes to 24 hours (more preferably from 1 to 10 hours) will usually suffice.

The reaction of Step A2(2), which comprises the conversion of the nitro group represented by R^{3a} in the compound of formula (Ic) into an amino group is preferably effected, in an inert solvent and in the presence of an acid, by reaction of a nitro compound of formula (Ic) in which R^{3a} represents a nitro group with a reducing agent, for example a metal powder. Suitable reducing metal powders include powders of iron, tin or zinc. Of these, iron or tin powder is preferred.

Suitable acids include: mineral acids, such as hydrochloric acid or sulfuric acid; and organic acids, such as acetic acid, trifluoroacetic acid, methanesulfonic acid or p-toluenesulfonic acid. Of these, hydrochloric acid or acetic acid is preferred.

The reaction is normally and preferably effected in the presence of a solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or on the reagents involved and that it can dissolve the reagents, at least to some extent. Examples of suitable solvents include: water; ethers, such as diethyl ether, tetrahydrofuran or dioxane; alcohols, such as methanol or ethanol; the acid employed for the reaction, as mentioned above; or a mixture of any two or more of these

solvents. Of these, we prefer to use a mixture of water with an acid.

The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. In general, we find it convenient to carry out the reaction at a temperature of from -10°C. to 100°C. (more preferably from 0°C. to 50°C.), although this may vary depending on the nature of the starting material of formula (Ic) and on the solvent employed. The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the reagents and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from 15 minutes to 20 hours (more preferably from 30 minutes to 10 hours) will usually suffice. If this reaction is carried out in an organic acid and in the presence of one of the acid anhydrides mentioned later in connection with the reaction of Step A2(3), this reaction affords an amino-acylated compound.

Conversion of the nitro group into an amino group can be also conducted in a similar manner to Step C2(4) of Reaction Scheme C as described hereafter, and, in this case, any nitro group contained in R^1 is converted into an amino group at the same time.

Alkylation, aralkylation or acylation of the amino group can be conducted by reacting an amino compound of formula (Ic) in which R^3 represents an amino group with a corresponding alkyl halide, aralkyl halide, acyl halide or acid anhydride [for example: an alkyl halide having from 1 to 4 carbon atoms; an alkyl halide having from 1 to 4 carbon atoms which is substituted by an alkoxy group having from 1 to 4 carbon atoms, by an alkanoyloxy group having from 1 to 6 carbon atoms or by an arylcarbonyloxy group having from 6 to 10 carbon atoms in the aryl moiety; an aralkyl halide having from 7 to 14 carbon atoms; an alkanoyl halide having from 2 to 18 carbon atoms or a mixed acid anhydride of a corresponding acid with formic acid; an alkenoyl halide having from 3 to 6 carbon atoms; a cycloalkane carbonyl halide having from 3 to 7 carbon atoms in the cycloalkane moiety; an arylcarbonyl halide having from 6 to 10 carbon atoms in the aryl moiety; an alkoxy-carbonyl halide having from 1 to 4 carbon atoms in the alkoxy moiety; an alkyl carbonate anhydride having from 1 to 4 carbon atoms in the alkyl moiety; an aralkyloxy carbonyl halide having from 7 to 14 carbon atoms in the aralkyl moiety; a phthalidyl halide; or a (5-methyl or 5-phenyl-2-oxo-1,3-dioxolen-4-yl)methyl halide, all as exemplified above in relation to Step A2(1)]. This reaction normally and preferably takes place in an inert solvent and in the presence of a base. If it is desired to prepare a mono-alkylamino compound having from 1 to 4 carbon atoms, we prefer to use about an equimolar amount of an alkyl halide having from 1 to 4 carbon atoms with respect to the compound of formula (I); on the other hand, the desired compound is a di-alkylamino compound having from 1 to 4 carbon atoms in each alkyl moiety, it is preferred to use more than about 2 moles of an alkyl halide having from 1 to 4 carbon atoms per mole of the compound of formula (I).

The reaction is essentially the same as that employed in Step A1, and may be carried out using the reaction conditions, base and solvent as described above in relation to that reaction.

After completion of the reaction or any of the reactions described above, the desired compound can be

obtained from the reaction mixture by conventional means. For example, one suitable recovery procedure comprises: filtering off any insoluble matter; adding water to the filtrate; if necessary, neutralizing the resulting mixture; extracting it with a water-immiscible organic solvent, such as ethyl acetate; drying it; and distilling off the solvent. If necessary, the product thus obtained can be further purified by conventional means, such as recrystallization or the various chromatography techniques, for example preparative thin layer chromatography or column chromatography, notably column chromatography.

A salt of the compound of formula (I) can be prepared by conventional means, as is well known in the art. For example, the compound of formula (I) is treated with an acid, such as hydrochloric acid or maleic acid, in an inert solvent, such as diethyl ether or diisopropyl ether, and the separated crystals are recovered by filtration.

An optically active compound of formula (I) can be prepared by using a corresponding optically active benzyl halide of formula (II) as the starting material, or by optical resolution of a racemic compound of formula (I) by conventional means, such as fractional crystallization or liquid chromatography.

The condensed hydropyridyl compound of formula (IV), used as one of the starting materials, is known or may easily be prepared by any known method [for example, M. Podesta et al., *Eur. J. Med. Chem. - Chim. Ther.* 9 (5), 487-490 (1974); and Japanese Patent Kokai Application No. Sho 61-246186]. Compounds of formula (IV) having a nitro group as the group R^{3a} are known or can be prepared as follows:

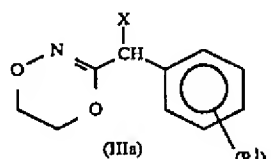
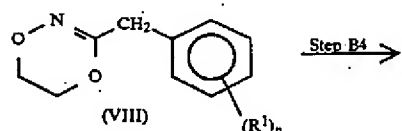
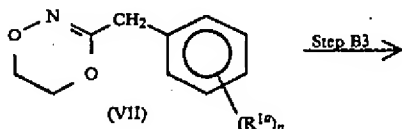
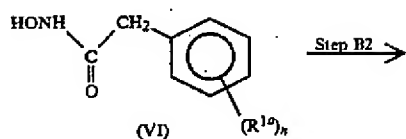
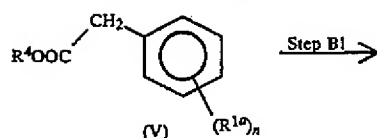
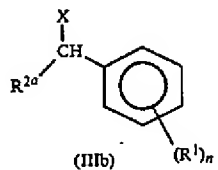
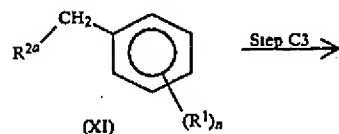
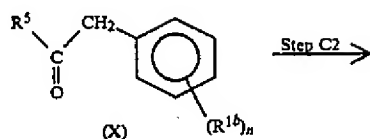
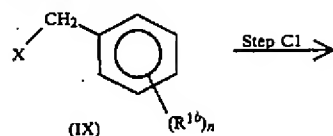
The imino group in a compound corresponding to the compound of formula (IV), but in which the group R^{3a} is a hydrogen atom [which can easily be prepared by any known method (for example as described in Japanese Patent Kokai Application No. Sho 62-103088)] is protected. The protecting reaction can be conducted in a similar way to that described in Step A2(3) of Reaction Scheme A, above. The protecting group may be, for example, an acyl group, such as an alkanoyl group having from 1 to 18 carbon atoms as exemplified above. The protected compound is then allowed to react in an inert solvent (which may be, for example, a fatty acid, such as acetic acid or propionic acid, or acid anhydride, such as acetic anhydride or propionic anhydride, or a mixture of any two or more thereof) with a nitrating agent (such as fuming nitric acid or anhydrous nitric acid) at a suitable temperature, for example from 0°C. to 50°C. , for a period of, for example, from 15 minutes to 5 hours, and is finally treated with an acid (such as aqueous hydrochloric acid or aqueous sulfuric acid) at a suitable temperature, for example from 20°C. to 100°C. , for a period of, for example, from 15 minutes to 5 hours to remove the protecting group.

The compound of formula (III), which is the other starting material, can easily be prepared, for example by the processes shown below in Reaction Schemes B, C, D and E.

Reaction Scheme B:

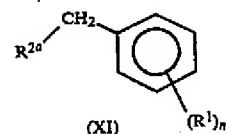
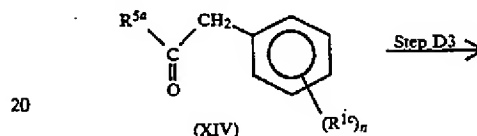
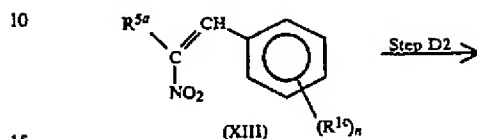
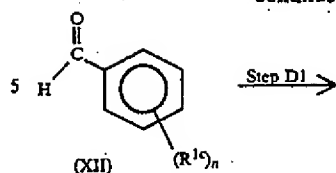
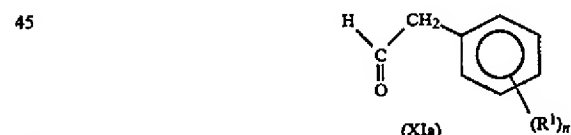
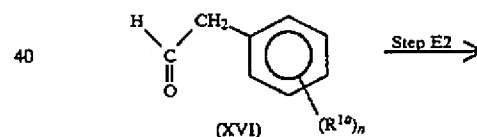
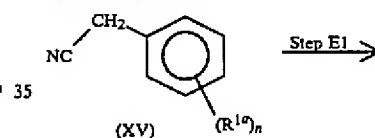
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-continued

Reaction Scheme C:Reaction Scheme D:

30

-continued

Reaction Scheme E:

In these formulae, R¹, X and n are as defined above.

R^{1a} represents a hydrogen atom, an alkyl group having from 1 to 4 carbon atoms, a halogen atom, a haloalkyl group having from 1 to 4 carbon atoms and at least one halogen atom, a hydroxy group, an alkoxy group having from 1 to 4 carbon atoms, a haloalkoxy group having from 1 to 4 carbon atoms and at least one halogen atom, an alkylthio group having from 1 to 4 carbon atoms and at least one halogen atom, a haloalkylthio group having from 1 to 4 carbon atoms and at least one halogen atom, an amino group, an protected alkanoyl group having from 1 to 5 carbon atoms in the alkanoyl part, a protected haloalkanoyl group having from 2 to 5 carbon atoms and at least one halogen atom in the haloalkanoyl part, a carbamoyl group, a nitro group, an alkanesulfonyl group having from 1 to 4 carbon atoms, a haloalkanesulfonyl group having from 1 to 4 carbon atoms and at least one halo-

gen atom, or a sulfamoyl group. That is, it represents the same groups as does R¹, other than the cyano, carboxy and alkoxy, carbonyl, and the alkanoyl groups and the haloalkanoyl groups are protected.

R^{1b} represents a hydrogen atom, an alkyl group having from 1 to 4 carbon atoms, a halogen atom, a haloalkyl group having from 1 to 4 carbon atoms and at least one halogen atom, a protected hydroxy group, an alkoxy group having from 1 to 4 carbon atoms, a haloalkoxy group having from 1 to 4 carbon atoms and at least one halogen atom, an alkylthio group having from 1 to 4 carbon atoms, a haloalkylthio group having from 1 to 4 carbon atoms and at least one halogen atom, an protected alkanoyl group having from 1 to 5 carbon atoms in the alkanoyl part, a protected haloalkanoyl group having from 2 to 5 carbon atoms and at least one halogen atom in the haloalkanoyl part, a nitro group, an alkanesulfonyl group having from 1 to 4 carbon atoms, or a haloalkanesulfonyl group having from 1 to 4 carbon atoms and at least one halogen atom. That is, it represents the same groups as does R¹, other than the amino, cyano, carboxy, carbamoyl, sulfamoyl and alkoxycarbonyl groups, and the alkanoyl groups, the haloalkanoyl groups and the hydroxy groups are protected.

R^{1c} represents the same groups as are defined above for R¹, except that the alkanoyl group having from 1 to 5 carbon atoms and the haloalkanoyl group having from 2 to 5 carbon atoms are protected.

R^{2a} represents the same groups as are defined above for R², other than the dihydrodioxazinyl group.

R⁴ represents an alkyl group having from 1 to 4 carbon atoms.

R⁵ represents a hydrogen atom, an alkyl group having from 1 to 9 carbon atoms, a substituted alkyl group which has from 1 to 9 carbon atoms and which is substituted by at least one substituent selected from the group consisting of substituents A, defined above, an alkenyl group having from 2 to 5 carbon atoms, a substituted alkenyl group which has from 2 to 5 carbon atoms and which is substituted by at least one substituent selected from the group consisting of substituents A, defined above, a cycloalkyl group having from 3 to 7 carbon atoms, a substituted cycloalkyl group which has from 3 to 7 carbon atoms and which is substituted by at least one substituent selected from the group consisting of substituents A, defined above, or a substituted phenyl group having at least one substituent selected from the group consisting of substituents B, defined above, and provided that any hydroxy group in substituents A is protected. That is, it represents any of the groups (other than the dihydrodioxazinyl group) defined above for R², but without the terminal carbonyl group.

R^{5a} represents any of the groups represented by R⁵, except that the hydroxy group of substituent A need not be protected.

There is no particular limitation on the nature of the protecting group for the alkanoyl group having from 1 to 5 carbon atoms or the haloalkanoyl group having from 2 to 5 carbon atoms, and any such group commonly used for the protection of aldehydes and ketones in the field of organic chemistry. Examples include an acetal or ketal containing a carbonyl moiety as shown in the following formula:



in which R⁶ and R⁷ are the same or different and each represents an alkyl group having from 1 to 4 carbon atoms (such as a methyl, ethyl, propyl, isopropyl or butyl group) or R⁶ and R⁷ together form an alkylene group having 2 or 3 carbon atoms (such as an ethylene or trimethylene group). We prefer an acetal or ketal in which R⁶ and R⁷ are each a methyl or ethyl group, or R⁶ and R⁷ together form an ethylene or trimethylene group.

The nature of the hydroxy-protecting groups which may be employed in this reaction is not critical and any hydroxy-protecting group known for use in this type of reaction may equally be employed here. Examples of such groups include groups derived from the cyclic ethers, such as the tetrahydropyranyl or tetrahydrofuranlyl group.

In Reaction Scheme B, a compound of formula (IIIa) is prepared; this is a compound of formula (III) in which R² is a dihydrodioxazinyl group.

In Step B1 of this Reaction Scheme, a compound of formula (VI) is prepared by reacting a compound of formula (V) with hydroxylamine or with a mineral acid salt of hydroxylamine (such as the hydrochloride or the sulfate) in an inert solvent (for example, an alcohol such as methanol or ethanol) and in the presence of a base (for example, an alkali metal alkoxide such as sodium methoxide, sodium ethoxide or potassium t-butoxide) at a suitable temperature, preferably from 0° C. to 150° C. (more preferably from about room temperature to 100° C.) for a suitable period, preferably from 1 to 24 hours (more preferably from 2 to 15 hours).

In Step B2 of this Reaction Scheme, a compound of formula (VII) is prepared by reacting a compound of formula (VI) with a compound of formula (XVIII):



in which X^a and X^b are the same or different and each represents a halogen atom. The reaction is normally and preferably effected in the presence of a solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or on the reagents involved, and that it can dissolve the reagents, at least to some extent. Examples of suitable solvents include: water, and alcohols, such as methanol or ethanol. The reaction is also preferably effected in the presence of a base, the nature of which is also not critical to the present invention. Examples of such bases include: alkali metal carbonates, such as sodium carbonate or potassium carbonate; and alkali metal hydroxides, such as sodium hydroxide or potassium hydroxide. The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the present invention. In general, we find it convenient to carry out the reaction at a temperature of from 0° C. to 200° C. (more preferably at a temperature from about room temperature to 150° C.). The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the reagents and solvent employed. However, provided that the reaction

is effected under the preferred conditions outlined above, a period of from 1 to 24 hours (more preferably from 2 to 15 hours) will usually suffice.

Step B3 of this Reaction Scheme is optional to give a compound of formula (VIII), and may consist of one or more of the following reactions:

- (1) Removal of the alkanoyl or haloalkanoyl-protecting group contained in R^{1a};
- (2) Conversion of the halogen atom contained in R^{1a} into a cyano group;
- (3) Conversion of the halogen atom contained in R^{1a} into a carboxy group, followed, if desired, by conversion of the carboxy group into an alkoxy-carbonyl group having from 1 to 4 carbon atoms in the alkoxy moiety.

In Step B3(1) of this Reaction Scheme, removal of the alkanoyl- or haloalkanoyl-protecting group can be effected by conventional means commonly employed in the field of organic chemistry. For example, if the protecting group is an acetal or a ketal, a corresponding compound of formula (VII) is reacted with an acid (for example, a mineral acid, such as hydrochloric acid, sulfuric acid or nitric acid; or an organic acid, such as acetic acid, trifluoroacetic acid, methanesulfonic acid or p-toluenesulfonic acid). The reaction is normally and preferably effected in the presence of a solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or on the reagents involved and that it can dissolve the reagents, at least to some extent. Examples of suitable solvents include: water and alcohols, such as methanol or ethanol. The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. In general, we find it convenient to carry out the reaction at a temperature of from 0° C. to 100° C. (more preferably at a temperature from about room temperature to 50° C.). The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the reagents and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from 10 minutes to 5 hours (more preferably from 30 minutes to 2 hours) will usually suffice.

Conversion of a halogen atom into a cyano group in Step B3(2) of this Reaction Scheme is preferably effected by reacting the corresponding compound of formula (VII) with a metal cyanide, such as sodium cyanide, potassium cyanide or copper cyanide. The reaction is normally and preferably effected in the presence of a solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or on the reagents involved and that it can dissolve the reagents, at least to some extent. Examples of suitable solvents include: amides, such as dimethylformamide or dimethylacetamide; and ethers, such as diethyl ether or tetrahydrofuran. The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. In general, we find it convenient to carry out the reaction at a temperature of from 0° C. to 200° C. (more preferably at a temperature from about room temperature to 150° C.). The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the reagents and solvent employed. However, provided that the reaction is effected under

the preferred conditions outlined above, a period of from 1 to 24 hours (more preferably from 2 to 15 hours) will usually suffice.

Conversion of the halogen atom into a carboxy group in Step B3(3) of this Reaction Scheme is preferably effected by reacting the corresponding compound of formula (VII) with magnesium. The reaction is normally and preferably effected in the presence of a solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or on the reagents involved and that it can dissolve the reagents, at least to some extent. Examples of suitable solvents include: ethers, such as diethyl ether or tetrahydrofuran. The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. In general, we find it convenient to carry out the reaction at a temperature of from 0° C. to 150° C. (more preferably at a temperature from about room temperature to 100° C.). The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the reagents and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from 30 minutes to 24 hours (more preferably from 1 to 10 hours) will usually suffice. The resulting Grignard reagent is then reacted with carbon dioxide gas at a temperature from, for example, 0° C. to 150° C. (more preferably at a temperature from about room temperature to 100° C.) for a suitable period, for example from 30 minutes to 24 hours (more preferably from 1 to 10 hours).

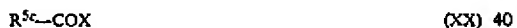
Conversion of the resulting carboxy group into an alkoxy-carbonyl group having from 1 to 4 carbon atoms can, if desired, be conducted by reacting the corresponding carboxylic acid with an alcohol having from 1 to 4 carbon atoms, such as methanol, ethanol, propanol, isopropanol or butanol, in the presence of an acid (for example, a mineral acid, such as hydrochloric acid, sulfuric acid or nitric acid; or an organic acid, such as acetic acid, trifluoroacetic acid, methane sulfonic acid or p-toluenesulfonic acid). The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. In general, we find it convenient to carry out the reaction at a temperature of from 0° C. to 100° C. (more preferably at a temperature from about room temperature to 50° C.). The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the reagents and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from 30 minutes to 5 hours (preferably from 1 to 2 hours) will usually suffice. Rather than using any additional solvent, this reaction is usually carried out by using as the solvent a large excess of the alcohol having from 1 to 4 carbon atoms, which is one of the reagents.

In Step B4, a compound of formula (IIIa) is prepared by reacting a compound of formula (VIII) with a halomide, such as N-chlorosuccinimide, N-bromosuccinimide or N-iodosuccinimide in the presence of a radical initiator, such as benzoyl peroxide, or by reacting said compound of formula (VIII) with a halogen, such as chlorine, bromine or iodine, in an inert solvent (for example, a halogenated hydrocarbon, preferably a halogenated aliphatic hydrocarbon, such as methylene chloride, chloroform or carbon tetrachloride). The reaction can

take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. In general, we find it convenient to carry out the reaction at a temperature of from 0° C. to 100° C. (more preferably at a temperature from about room temperature to 50° C.). The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the reagents and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from 30 minutes to 20 hours (more preferably from 1 to 15 hours) will usually suffice.

In Reaction Scheme C, a compound of formula (IIIb) is prepared. This is a compound of formula (III) in which R² is replaced by R^{2a}, that is any of the groups defined above for R² except a dihydrodioxazinyl group.

In Step C1 of this Reaction Scheme, a compound of formula (X) is prepared by reacting a compound of formula (IX) with magnesium in an inert solvent (for example, an ether, such as diethyl ether or tetrahydrofuran), to give a Grignard reagent. The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. In general, we find it convenient to carry out the reaction at a temperature of from 0° C. to 150° C. (more preferably at a temperature from about room temperature to 100° C.). The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the reagents and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from 30 minutes to 24 hours (more preferably from 1 to 10 hours) will usually suffice. The resulting Grignard reagent is then reacted with a compound of formula (XIX), (XX) or (XXI):



or



in which R⁵ and X are as defined above; R^{5b} represents any of the groups defined for R⁵, except a group having a cyano substituent; and R^{5c} represents any of the groups defined for R⁵, except a hydrogen atom. The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. In general, we find it convenient to carry out the reaction at a temperature of from 0° C. to 150° C. (more preferably at a temperature from about room temperature to 100° C.). The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the reagents and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from 30 minutes to 24 hours (more preferably from 1 to 10 hours) will usually suffice.

Step C2 of Reaction Scheme C comprises one or more of the following optional reactions:

- (1) Removal of the alkanoyl or haloalkanoyl-protecting group contained in R^{1b};
- (2) Removal of the hydroxy-protecting group contained in R^{1b}, R⁵ etc;

- (3) Conversion of the halogen atom contained in R^{1b} into a cyano group, and then optionally into a carbamoyl group, and then optionally into a carboxy, and finally optionally into an alkoxy-carbonyl group having from 1 to 4 carbon atoms in the alkoxy moiety;

- (4) Conversion of the nitro group contained in R^{1b} into an amino group; and

- (5) Conversion of the alkylthio group contained in R^{1b} into a sulfamoyl group.

Removal of the alkanoyl or haloalkanoyl-protecting group in Step C2(1) and removal of the hydroxy-protecting cyclic ether group in Step C2(2) can be conducted in a similar way to that in Step B3(1) of Reaction Scheme B, as described above.

Conversion of the halogen atom into a cyano group in Step C2(3) can be conducted in a similar way to that in Step B3(2) of Reaction Scheme B, as described above. In this reaction, it is preferred not to use as the starting material a compound of formula (X) containing a halogen atom in the substituent of R⁵. If a compound containing a halogen atom in the substituent R⁵ is used, conversion of this halogen atom into a cyano group is also possible.

Successive conversion of the cyano group into carbamoyl and carboxy groups can be conducted by reaction of a corresponding compound of formula (X) with an aqueous mineral acid (such as aqueous sulfuric acid, aqueous hydrochloric acid or aqueous nitric acid). The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. In general, we find it convenient to carry out the reaction at a temperature of from 0° C. to 200° C. (more preferably at a temperature from about room temperature to 100° C.). The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the reagents and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from 1 to 24 hours (more preferably from 2 to 15 hours) will usually suffice. In this reaction, it is possible to choose whether the carbamoyl or the carboxy compound will be obtained by adjusting the acid concentration. For example, the carbamoyl compound can be obtained by reaction in about 90% sulfuric acid, and then it can be converted into the carboxy compound by reaction in about 60% sulfuric acid.

Conversion of the carboxy group into an alkoxy-carbonyl group having from 1 to 4 carbon atoms in the alkoxy moiety can be conducted in a similar way to that described in Step B3(3) of Reaction Scheme B, as described above.

Conversion of the nitro group into an amino group in Step C2(4) can be conducted by reacting the corresponding compound of formula (X) with hydrogen gas (preferably at from 1 atmosphere to 5 atmospheres) in an inert solvent (for example, an alcohol, such as methanol or ethanol) and in the presence of a reducing catalyst (such as Raney-nickel, palladium-on-carbon or platinum oxide). The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. In general, we find it convenient to carry out the reaction at a temperature of from 0° C. to 150° C. (preferably at room temperature to 100° C.). The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the reagents and

solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from 30 minutes to 24 hours (more preferably from 1 to 10 hours) will usually suffice.

Conversion of the alkylthio group into a sulfamoyl group in Step C2(5) can be conducted by reacting a corresponding compound of formula (X) with a halogenating agent (such as chlorine or bromine) in an inert solvent (for example, water, an organic acid, such as acetic acid or propionic acid or a mixture of any two or more thereof), to give a sulfonyl halide. The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. In general, we find it convenient to carry out the reaction at a temperature of from -10°C. to 100°C. (more preferably at from 5°C. to 50°C.). The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the reagents and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from 30 minutes to 24 hours (more preferably from 1 to 10 hours) will usually suffice. The resulting sulfonyl halide is then reacted with ammonia in an inert solvent (for example, water or an alcohol, such as methanol or ethanol) at, for example, from 0°C. to 100°C. (more preferably at room temperature to 50°C.) for a suitable period, for example from 30 minutes to 24 hours (more preferably from 1 to 10 hours).

In Step C3 of Reaction Scheme C, a compound of formula (IIIb) is prepared by halogenation of the compound of formula (XI) prepared in Step C2. This reaction is essentially the same as that described in Step B4 of Reaction Scheme B, and may be carried out using the same reagents and reaction conditions.

Reaction Scheme D provides an alternative route for preparing the compound of formula (XI), which is also prepared in Step C2 of Reaction Scheme C.

In Step D1 of Reaction Scheme D, a compound of formula (XIII) is prepared by reacting a compound of formula (XII) with a compound of formula (XXII):



in which R^{5a} is as defined above. The reaction is normally and preferably effected in the presence of a solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or on the reagents involved and that it can dissolve the reagents, at least to some extent. Examples of suitable solvents include organic acids, such as acetic acid or propionic acid. The reaction is also normally and preferably effected and in the presence of a base, for example, an ammonium salt of an organic acid, such as ammonium acetate, ammonium propionate or ammonium benzoate. The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. In general, we find it convenient to carry out the reaction at a temperature of from about room temperature to 200°C. (more preferably at from 50°C. to 150°C.). The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the reagents and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from 1 to 24 hours (more preferably from 2 to 15 hours) will usually suffice.

In Step D2 of Reaction Scheme D, a compound of formula (XIV) is prepared by reacting a compound of formula (XIII) with a reducing agent (such as zinc or iron) in an inert solvent (for example, an organic acid, such as acetic acid or propionic acid) and in the presence of water. The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. In general, we find it convenient to carry out the reaction at a temperature of from about room temperature to 250°C. (more preferably at from 50°C. to 150°C.). The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the reagents and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from 30 minutes to 24 hours (more preferably from 1 to 10 hours) will usually suffice.

Step D3 of this Reaction Scheme is optional and comprises removal of the alkanoyl- or haloalkanoyl protecting group contained in R^{1c} . The removal reaction is essentially the same reaction as that employed in Step B3 of Reaction Scheme B, and may be carried out employing the same reagents and reaction conditions.

Reaction Scheme E provides an alternative route for preparing a compound of formula (XI), which is also prepared in Step C2 of Reaction Scheme C, when R^{2a} in the compound of formula (XI) is a formyl group, that is a compound of formula (XIa).

In Step E1 of Reaction Scheme E, a compound of formula (XVI) is prepared by reacting a compound of formula (XV) with a reducing agent [for example, an aluminum hydride, such as lithium tri(*t*-butoxy)aluminum hydride or lithium aluminum hydride]. The reaction is normally and preferably effected in the presence of a solvent. There is no particular restriction on the nature of the solvent to be employed, provided that it has no adverse effect on the reaction or on the reagents involved and that it can dissolve the reagents, at least to some extent. Examples of suitable solvents include ethers, such as diethyl ether or tetrahydrofuran. The reaction can take place over a wide range of temperatures, and the precise reaction temperature is not critical to the invention. In general, we find it convenient to carry out the reaction at a temperature of from -30°C. to 50°C. (more preferably at from 0°C. to room temperature). The time required for the reaction may also vary widely, depending on many factors, notably the reaction temperature and the nature of the reagents and solvent employed. However, provided that the reaction is effected under the preferred conditions outlined above, a period of from 1 to 24 hours (more preferably from 2 to 15 hours) will usually suffice.

Step E2 of Reaction Scheme E is optional and comprises one or more of the following reactions:

- (1) Removal of the alkanoyl or haloalkanoyl-protecting group contained in R^{1c} ;
- (2) Conversion of the halogen atom contained in R^{1a} into a cyano group, which may then, if desired, be converted into a carboxy group, which finally may, if desired, be converted into an alkoxycarbonyl group.

These reactions are essentially the same as those described above in relation to Step C2 of Reaction Scheme C, and may be carried out employing the same reagents and reaction conditions.

After completion of any of these reactions, the desired compound can be recovered from the reaction

mixture by conventional means. For example, insoluble matter, if any, is filtered off, and, if the reaction solution is acidic or alkaline, the solution is neutralized. The desired product can then be recovered by distilling off the solvent, or by adding water, extracting the resulting mixture with a water-immiscible organic solvent, such as ethyl acetate, drying the extract, and then distilling off the solvent. If necessary, the product thus obtained can be further purified by conventional means, such as recrystallization or the various chromatography techniques, for example preparative thin layer chromatography or column chromatography, notably column chromatography.

Alternatively, when the desired compound is a carboxylic acid derivative, it may be recovered from the reaction medium by the following procedure: making the reaction solution alkaline; extracting the resulting mixture with a water-immiscible organic solvent, such as ethyl acetate; neutralizing the aqueous layer; extracting the desired compound with a water-immiscible organic solvent, such as ethyl acetate; drying the extract; and then distilling off the solvent.

The compounds of the present invention prepared as described above may be converted to acid addition salts and/or to complexes with metal ions by methods well known in the art.

BIOLOGICAL ACTIVITY

The compounds of formula (I) and their tautomers, salts and complexes of the present invention have an excellent inhibitory activity against blood platelet aggregation, and are therefore very useful for prevention and therapy of thrombosis and embolism. These activities are demonstrated by the following Test Examples, which employ techniques well recognized in the art to provide a model of such activity in humans and other mammals.

TEST EXAMPLE 1

Prolongation of Bleeding Time in mice

Male mice of the ICR strain (supplied by Japan Charles River Inc.) were divided into groups of 10 each for the test. A sample of the drug to be tested was suspended in a 5% w/v aqueous solution of gum arabic, and administered orally to the mice at a dose of 3 mg/kg for 3 successive days, namely 48 hours, 24 hours and 4 hours before the bleeding test. For the test, each of the mice was fixed by use of conventional apparatus, and the tail was cut 5 mm from the end. The last 2 cm of the tail was soaked in physiological saline kept warm at 37° C. The time at which bleeding was observed to cease for a successive 15 seconds was regarded as the point at which bleeding stopped, and the time between cutting the tail until the point when bleeding stopped was recorded as the bleeding time. The bleeding time was observed for a maximum of 5 minutes, and, even if bleeding continued for longer than 5 minutes, the bleeding time was reported as 5 minutes (300 seconds). The results are shown in Table 2. The test was carried out using certain of the compounds of the present invention, as well as with two prior art compounds.

Each of the compounds of the present invention is identified in the Table by the number assigned to it in the foregoing Table 1 and by the number of the Example hereafter which illustrates its preparation. The prior art compounds are identified as follows:

Compound A: 5-(2-chlorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;

Compound B: 5-(2-chloro- α -methoxycarbonylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine.

TEST EXAMPLE 2

Inhibition of Blood Platelet Aggregation

Female rats of the SD strain (supplied by Japan Charles River Inc.) were divided into groups of 4 each for the test. A sample of the drug to be tested was suspended in a 5% w/v aqueous solution of gum arabic, and administered orally to the rats 4 hours before the test. As a control, a 5% w/v aqueous solution of gum arabic was administered to a control group of rats, without any test drug. Blood platelet aggregation was tested according to the method of P. Lumley and P. P. A. Humphrey [J. Pharmacol. Methods 6, 153-166 (1981)] with a partial modification. From the abdominal aorta of the anesthetized rat, 5.4 ml of a blood sample was taken in 0.6 ml of a 3.8% (w/v) sodium citrate solution serving as an anticoagulant. The resulting citrate-containing blood samples were poured into cuvettes, with 1.2 ml in each cuvette, and stirred (1000 rpm) at 37° C. After preliminary heating for 2 minutes, 0.3 ml of the blood sample was taken out of each of the cuvettes, and the blood platelet count was measured by means of an automatic blood cell counter (E-4000, Sysmex); this was regarded as the blood platelet count before addition. 0.9 ml of the blood sample in the cuvette was then mixed with 0.1 ml of a 0.05M adenosine diphosphate (ADP) solution or with 0.1 ml of a collagen suspension (0.06 mg/ml), to induce blood platelet aggregation. Two minutes after addition of the ADP, or 4 minutes after addition of the collagen, 0.3 ml of the blood sample was taken and the blood platelet count was measured; this was regarded as the blood platelet count after addition. The blood platelet aggregation rate was calculated from the following equation.

$100 \times (\text{blood platelet count before addition} - \text{blood platelet count after addition}) / \text{blood platelet count before addition}$

The inhibitory effect was calculated as the percent inhibition of the treated groups as compared with the control groups. The results are reported in Table 2.

TABLE 2

Ex. No.	Cpd. No.	Test Ex. 1 Bleeding time (hours)		Test Ex. 2 % Inhibition	
		3 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg
5	60	2.20	—	74.2	100
6	19	2.13	—	29.3	97.8
12	59	>2.75	57.1	98.1	—
20	235	>2.75	98.8	—	—
22	233	2.30	—	—	98.9
23	190	>2.75	100	—	—
25	194	>2.75	100	—	—
26	196	>2.75	97.6	—	—
Compound A		1.00	—	—	3.7*
Compound B		1.80	—	25.7	98.8

*at a dose of 30 mg/kg.

For therapeutic or prophylactic use, the compounds of the present invention may be administered by themselves or in admixture with any one or more conventional carriers, diluents or additives. Administration may be by any convenient route, for example orally or parenterally, and the formulation will be chosen having regard to the intended route of administration. The

compounds may, for example, be administered in the form of powders, granules, tablets, capsules and injections. The dosage may vary depending upon the severity and nature of the disorder, as well as the symptoms, age and body weight of the patient and the chosen route of administration; however, in the case of oral administration, we would normally suggest a dose of from 1 to 1000 mg, more preferably from 10 to 500 mg, if administered orally, or a dose of from 0.5 to 500 mg, more preferably from 5 to 250 mg, if administered intravenously. The compound may be administered in single or divided doses, e.g. from 1 to 3 times a day depending on the symptoms.

The preparation of the compounds of the present invention is further illustrated by the following non-limiting Examples, whilst the preparation of certain of the starting materials used in these Examples is illustrated by the subsequent Preparations.

EXAMPLE 1

5-(2-Chloro- α -trifluoroacetylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (Compound No. 113)

10 ml of methylene chloride were added to 0.39 g (2.6 mmole) of 4,5,6,7-tetrahydrothieno[3,2-c]pyridine hydrochloride and 0.28 g (2.6 mmole) of sodium carbonate, and then a solution of 0.67 g (2.2 mmole) of 2-chloro- α -trifluoroacetylbenzyl bromide in 10 ml of methylene chloride was slowly added to the resulting mixture, whilst stirring at room temperature. The mixture was then stirred at room temperature for 3 hours. At the end of this time, 200 ml of ethyl acetate were added to the reaction mixture, and the organic layer was separated, washed with a saturated aqueous solution of sodium chloride and dried over anhydrous magnesium sulfate. The solvent was removed by distillation under reduced pressure, and the resulting residue was subjected to silica gel column chromatography, using a 100:4 by volume mixture of toluene and ethyl acetate as the eluent, to give 0.31 g of the title compound as a colorless oil.

Infrared Absorption Spectrum (thin film) ν_{\max} cm⁻¹: 1685, 1705.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 2.90-3.04 (2H, multiplet); 3.90 (1H, triplet, J=6.0 Hz); 4.01 (1H, triplet, J=6.0 Hz); 5.51 (1H, doublet, J=7.3 Hz); 5.58 (1H, doublet, J=7.3 Hz); 6.82 (1H, doublet, J=5.4 Hz); 7.19 (2H, doublet, J=5.4 Hz); 7.36-7.58 (4H, multiplet).

Mass spectrum (CI, m/z): 360 (M⁺+1). Here and hereafter, in the mass spectra, "CI" means "chemical ionization".

EXAMPLE 2

5-[2-Chloro- α -(5,6-dihydro-1,4,2-dioxazin-3-yl)benzyl]-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and its hydrochloride (Compound No. 3)

2(a) Following a procedure similar to that described in Example 1, except that an equivalent amount of 2-chloro- α -(5,6-dihydro-1,4,2-dioxazin-3-yl)benzyl bromide (prepared as described in Preparation 18) was used in place of the 2-chloro- α -trifluoroacetylbenzyl bromide, the title compound was obtained as a colorless oil in a yield of 77%.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 2.77-2.94 (4H, multiplet); 3.63 (1H, doublet, J=14.4 Hz); 3.79 (1H, doublet, J=14.4 Hz); 3.96-4.02 (1H, multiplet); 4.08-4.14 (1H, multiplet); 4.27-4.32 (1H, multiplet); 4.36-4.42 (1H, multiplet); 4.75 (1H,

singlet); 6.70 (1H, doublet, J=5.4 Hz); 7.07 (1H, doublet, J=5.4 Hz); 7.20-7.90 (4H, multiplet).

Mass spectrum (CI, m/z): 349 (M⁺+1).

2(b) 2.7 g of the title compound obtained as described in step (a) above were dissolved in 100 ml of diethyl ether, and hydrogen chloride gas was blown into the resulting solution at room temperature. The crystals which precipitated were collected to obtain 2.3 g of the hydrochloride of the title compound as a colorless powder, melting at 104°-107° C.

Elemental analysis: Calculated for C₁₇H₁₇ClN₂O₂S.HCl.3/2H₂O: C, 49.52%; H, 5.13%; N, 6.80%. Found: C, 49.81%; H, 4.73%; N, 6.56%.

EXAMPLE 3

5-[2-Fluoro- α -(5,6-dihydro-1,4,2-dioxazin-3-yl)benzyl]-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and its hydrochloride (Compound No. 2)

3(a) Following a procedure similar to that described in Example 1, except that an equivalent amount of 2-fluoro-(5,6-dihydro-1,4,2-dioxazin-3-yl)benzyl bromide (prepared as described in Preparation 19) was used in place of the 2-chloro- α -trifluoroacetylbenzyl bromide, the title compound was obtained as a colorless oil in a yield of 50%.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 2.73-2.98 (4H, multiplet); 3.63 (1H, doublet, J=13.8 Hz); 3.79 (1H, doublet, J=13.8 Hz); 3.95-4.18 (2H, multiplet); 4.23-4.45 (1H, multiplet); 4.61 (1H, singlet); 6.70 (1H, doublet, J=5.4 Hz); 7.09 (1H, doublet, J=5.4 Hz); 7.20-7.80 (4H, multiplet).

Mass spectrum (CI, m/z): 333 (M⁺+1).

3(b) The procedure described in Example 2(b) was repeated, using the title compound as prepared in step (a) above, to obtain the hydrochloride of the title compound as a colorless powder, melting at 108°-112° C., in a yield of 81%.

Elemental analysis: Calculated for C₁₇H₁₇FN₂O₂S.HCl.H₂O: C, 52.78%; H, 5.21%; N, 7.24%. Found: C, 53.19%; H, 4.99%; N, 7.16%.

EXAMPLE 4

5-[2,6-Difluoro- α -(5,6-dihydro-1,4,2-dioxazin-3-yl)benzyl]-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (Compound No. 7)

Following a procedure similar to that described in Example 1, except that an equivalent amount of 2,6-difluoro- α -(5,6-dihydro-1,4,2-dioxazin-3-yl)benzyl bromide (prepared as described in Preparation 20) was used in place of the 2-chloro- α -trifluoroacetylbenzyl bromide, the title compound was obtained as a colorless powder, melting at 151°-153° C., in a yield of 8%.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 2.81-2.93 (4H, multiplet); 3.62 (1H, doublet, J=14.0 Hz); 3.79 (1H, doublet, J=14.0 Hz); 4.00-4.10 (2H, multiplet); 4.26-4.36 (2H, multiplet); 4.59 (1H, singlet); 6.70 (1H, doublet, J=5.4 Hz); 7.08 (1H, doublet, J=5.4 Hz); 7.20-7.80 (4H, multiplet).

Mass spectrum (CI, m/z): 351 (M⁺+1).

Elemental analysis: Calculated for C₁₇H₁₆F₂N₂O₂S: C, 58.27%; H, 4.60%; N, 8.00%. Found: C, 58.22%; H, 4.61%; N, 7.79%.

EXAMPLE 5

5-(2-Chloro- α -cyclopropylcarbonylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and its sulfate (Compound No. 60)

5(a) Following a procedure similar to that described in Example 1, except that an equivalent amount of 2-chloro- α -cyclopropylcarbonylbenzyl bromide was used in place of the 2-chloro- α -trifluoroacetylbenzyl bromide, the title compound was obtained as a yellow oil in yield of 66%.

Nuclear Magnetic Resonance Spectrum (CDCl_3) δ ppm: 0.78–0.90 (2H, multiplet); 0.96–1.06 (2H, multiplet); 2.15–2.29 (1H, multiplet); 2.83–2.94 (4H, multiplet); 3.56 (1H, doublet, $J=4.3$ Hz); 3.72 (1H, doublet, $J=4.3$ Hz); 5.06 (1H, singlet); 6.68 (1H, doublet, $J=4.9$ Hz); 7.06 (1H, doublet, $J=4.9$ Hz); 7.10–7.70 (4H, multiplet).

Mass spectrum (CI, m/z): 332 ($M^+ + 1$), 262.

5(b) A procedure similar to that described in Example 2(b) was repeated, using the title compound as prepared in step (a) above, except that concentrated sulfuric acid was added in place of blowing hydrogen chloride gas through the mixture, to obtain the sulfate of the title compound as white crystals, melting at 184° – 186° C., in a yield of 70%.

Elemental analysis: Calculated for $\text{C}_{18}\text{H}_{18}\text{ClNOS} \cdot \text{H}_2\text{SO}_4$: C, 50.28%; H, 4.69%; N, 3.26%; Found: C, 50.43%; H, 4.53%; N, 2.87%.

EXAMPLE 6

5-(2-Fluoro- α -propionylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and its maleate (Compound No. 19)

6(a) 1.85 g 11.13 mmole of 1-(2-fluorophenyl)-2-butanone (prepared as described in Preparation 9) were dissolved in 30 ml of carbon tetrachloride, and then a solution of 1.78 g of bromine in 15 ml of carbon tetrachloride was added dropwise to the resulting solution at room temperature over a period of 30 minutes. The resulting mixture was then stirred at room temperature for 5 hours, after which water was added to the reaction mixture. The reaction mixture was then extracted with chloroform, and the extract was dried over anhydrous magnesium sulfate. A crude 2-fluoro- α -propionylbenzyl bromide was obtained from this extract by removal of the solvent by evaporation under reduced pressure 1.95 g (11.13 mmole) of 4,5,6,7-tetrahydrothieno[3,2-c]pyridine hydrochloride, 3.38 g (24.45 mmole) of anhydrous potassium carbonate and 30 ml of dimethylformamide were added to the crude product thus obtained, and the resulting mixture was stirred at room temperature for 5 hours. At the end of this time, toluene was added to the reaction mixture, and after the insolubles had been removed by filtration, the filtrate was concentrated by evaporation under reduced pressure. The resulting residue was subjected to silica gel column chromatography, using a 19:1 by volume mixture of toluene and ethyl acetate as the eluent, to give 1.17 g of the title compound as a pale yellow oil.

Infrared Absorption Spectrum (thin film) ν_{\max} cm^{-1} : 1715.

Nuclear Magnetic Resonance Spectrum (CDCl_3) δ ppm: 1.03 (3H, triplet, $J=7.0$ Hz); 2.50 (2H, quartet, $J=7.0$ Hz); 2.80–2.95 (4H, multiplet); 3.53 (1H, doublet, $J=11.0$ Hz); 3.63 (1H, doublet, $J=11.0$ Hz); 4.75 (1H,

singlet); 6.67 (1H, doublet, $J=5.7$ Hz); 7.05 (1H, doublet, $J=5.7$ Hz); 7.10–7.55 (4H, multiplet).

Mass spectrum (CI, m/z): 304 ($M^+ + 1$), 246.

6(b) A procedure similar to that described in Example 2(b) was repeated, using the title compound prepared as described in step (a) above, except that maleic acid was added in place of blowing hydrogen chloride gas through the reaction mixture, to obtain the maleate of the title compound as a colorless powder, melting at 101° – 103° C., in a yield of 54%.

Elemental analysis: Calculated for $\text{C}_{17}\text{H}_{18}\text{FNOS} \cdot \text{C}_4\text{H}_4\text{O}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$: C, 58.86%; H, 5.41%; N, 3.27%; Found: C, 59.19%; H, 5.33%; N, 3.19%.

EXAMPLE 7

5-(α -Acetyl-2-chlorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and its hydrochloride (Compound No. 10)

7(a) Following a procedure similar to that described in Example 6, except that an equivalent amount of 1-(2-chlorophenyl)-2-propanone (prepared as described in Preparation 10) was used in place of the 1-(2-fluorophenyl)-2-butanone, the title compound was obtained as a pale yellow oil in a yield of 44%.

Infrared Absorption Spectrum (thin film) ν_{\max} cm^{-1} : 1715.

Nuclear Magnetic Resonance Spectrum (CDCl_3) δ ppm: 2.13 (3H, singlet); 2.70–2.95 (4H, multiplet); 3.50 (1H, doublet, $J=10.0$ Hz); 3.70 (1H, doublet, $J=10.0$ Hz); 4.93 (1H, singlet); 6.65 (1H, doublet, $J=5.7$ Hz); 7.05 (1H, doublet, $J=5.7$ Hz); 7.10–7.75 (4H, multiplet).

Mass spectrum (CI, m/z): 306 ($M^+ + 1$), 262.

7(b) A procedure similar to that described in Example 3(b) was repeated, using the title compound prepared as described in step (a) above, to obtain the hydrochloride of the title compound as a pale yellow powder, melting at 98° – 101° C., in a yield of 70%.

Elemental analysis: Calculated for $\text{C}_{16}\text{H}_{16}\text{ClNOS} \cdot \text{HCl} \cdot \frac{1}{2}\text{H}_2\text{O}$: C, 54.70%; H, 5.16%; N, 3.98%; Found: C, 55.09%; H, 4.97%; N, 3.80%.

EXAMPLE 8

5-(2-Chloro- α -propionylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and its hydrochloride (Compound No. 20)

8(a) Following a procedure similar to that described in Example 6, except that an equivalent amount of (2-chlorophenyl)-2-butanone (prepared as described in Preparation 11) was used in place of the 1-(2-fluorophenyl)-2-butanone, the title compound was obtained as a pale yellow oil in a yield of 32%.

Nuclear Magnetic Resonance Spectrum (CDCl_3) δ ppm: 1.05 (3H, triplet, $J=6.5$ Hz); 2.31–2.58 (2H, multiplet); 2.75–3.00 (4H, multiplet); 3.48 (1H, doublet, $J=14.5$ Hz); 3.68 (1H, doublet, $J=14.5$ Hz); 4.97 (1H, singlet); 6.65 (1H, doublet, $J=6.0$ Hz); 7.05 (1H, doublet, $J=6.0$ Hz); 7.10–7.65 (4H, multiplet).

Mass spectrum (CI, m/z): 320 ($M^+ + 1$).

8(b) A procedure similar to that described in Example 2(b) was repeated, using the title compound prepared as described in step (a) above, to obtain the hydrochloride of the title compound as a pale yellow powder, melting at 110° – 115° C., in a yield of 25%.

Elemental analysis: Calculated for $\text{C}_{17}\text{H}_{18}\text{ClNOS} \cdot \text{HCl} \cdot \text{H}_2\text{O}$: C, 54.55%; H, 5.92%; N, 3.74%; Found: C, 54.39%; H, 5.59%; N, 3.73%.

EXAMPLE 9

5-(2-Chloro- α -hexanoylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (Compound No. 125)

Following a procedure similar to that described in Example 6, except that an equivalent amount of 1-(2-chlorophenyl)-2-heptanone (prepared as described in Preparation 12) was used in place of the 1-(2-fluorophenyl)-2-butanone, the title compound was obtained as a yellow oil in a yield of 10%.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 0.90 (3H, triplet, $J=7.6$ Hz); 1.10-1.60 (6H, multiplet); 2.40 (2H, triplet, $J=8.0$ Hz); 2.75-3.00 (4H, multiplet); 3.50 (1H, doublet, $J=14.5$ Hz); 3.70 (1H, doublet, $J=14.5$ Hz); 5.00 (1H, singlet); 6.65 (1H, doublet, $J=6.0$ Hz); 7.05 (1H, doublet, $J=6.0$ Hz); 7.10-7.60 (4H, multiplet).

Mass spectrum (CI, m/z): 362 ($M^+ + 1$), 262.

EXAMPLE 10

5-(α -Acetyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and its maleate (Compound No. 9)

10(a) Following a procedure similar to that described in Example 6, except that an equivalent amount of 1-(2-fluorophenyl)-2-propanone was used in place of the 1-(2-fluorophenyl)-2-butanone, the title compound was obtained as a pale yellow oil in a yield of 55%.

Infrared Absorption Spectrum (thin film) ν_{max} cm⁻¹: 1715.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 2.18 (3H, singlet); 2.80-2.95 (4H, multiplet); 3.55 (1H, doublet, $J=12.0$ Hz); 3.65 (1H, doublet, $J=12.0$ Hz); 4.72 (1H, singlet); 6.65 (1H, doublet, $J=5.5$ Hz); 7.05 (1H, doublet, $J=5.5$ Hz); 7.10-7.55 (4H, multiplet).

Mass spectrum (CI, m/z): 290 ($M^+ + 1$), 246.

10(b) A procedure similar to that described in Example 2(b) was repeated, using the title compound prepared as described in step (a) above, except that maleic acid was added in place of blowing hydrogen chloride gas through the mixture, to obtain the maleate of the title compound as a pale yellow powder, melting at 104°-106° C., in a yield of 61%.

Elemental analysis: Calculated for C₁₆H₁₆FNOS.C₄H₄O₄. $\frac{1}{2}$ H₂O: C, 57.96%; H, 5.10%; N, 3.38%; Found: C, 58.36%; H, 4.94%; N, 3.39%.

EXAMPLE 11

5-(α -Cyclobutylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and its maleate (Compound No. 106)

11(a) Following a procedure similar to that described in Example 6, except that an equivalent amount of cyclobutyl-2-fluorobenzyl ketone (prepared as described in Preparation 13) was used in place of the 1-(2-fluorophenyl)-2-butanone, the title compound was obtained as a pale yellow oil in a yield of 24%.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 1.70-2.55 (6H, multiplet); 2.80-3.00 (4H, multiplet); 3.50 (1H, doublet, $J=11.0$ Hz); 3.62 (1H, doublet, $J=11.0$ Hz); 3.70-3.90 (1H, multiplet); 4.73 (1H, singlet); 6.65 (1H, doublet, $J=6.0$ Hz); 7.05 (1H, doublet, $J=6.0$ Hz); 7.10-7.50 (4H, multiplet).

Mass spectrum (CI, m/z): 330 ($M^+ + 1$), 246.

11(b) A procedure similar to that described in Example 2(b) was repeated, using the title compound prepared as described in step (a) above, except that maleic acid was added in place of blowing hydrogen chloride

gas through the mixture, to obtain the maleate of the title compound as a colorless powder, melting at 99°-104° C., in a yield of 57%.

Elemental analysis: Calculated for C₁₆H₁₆FNOS.C₄H₄O₄. $\frac{1}{2}$ H₂O: C, 60.78%; H, 5.54%; N, 3.08%; Found: C, 60.97%; H, 5.48%; N, 2.94%.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 1.70-2.30 (6H, multiplet); 3.10-3.30 (4H, multiplet); 3.68-3.82 (1H, multiplet); 4.30 (2H, broad singlet); 5.55 (1H, singlet); 6.30 (2H, singlet); 6.72 (1H, doublet, $J=6.5$ Hz); 7.20 (1H, doublet, $J=6.5$ Hz); 7.25-7.60 (4H, multiplet).

EXAMPLE 12

5-(α -Cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and its hydrochloride (Compound No. 59)

12(a) Following a procedure similar to that described in Example 6, except that an equivalent amount of cyclopropyl 2-fluorobenzyl ketone (prepared as described in Preparation 8) was used in place of the 1-(2-fluorophenyl)-2-butanone, the title compound was obtained as a colorless oil in a yield of 69%.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 0.78-0.90 (2H, multiplet); 0.98-1.11 (2H, multiplet); 2.22-2.34 (1H, multiplet); 2.72-2.98 (4H, multiplet); 3.58 (1H, doublet, $J=4.2$ Hz); 3.68 (1H, doublet, $J=4.2$ Hz); 4.85 (1H, singlet); 6.68 (1H, doublet, $J=4.9$ Hz); 7.06 (1H, doublet, $J=4.9$ Hz); 7.20-7.60 (4H, multiplet).

Mass spectrum (CI, m/z): 316 ($M^+ + 1$), 246.

12(b) A procedure similar to that described in Example 2(b) was repeated, using the title compound prepared as described in step (a) above, to obtain the hydrochloride of the title compound as white crystals, melting at 171°-173° C., in a yield of 75%.

Elemental analysis: Calculated for C₁₈H₁₈FNOS.HCl: C, 61.44%; H, 5.44%; N, 3.98%; Found: C, 61.37%; H, 5.74%; N, 3.85%.

EXAMPLE 13

5-(α -Butyryl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and its maleate (Compound No. 116)

13(a) Following a procedure similar to that described in Example 6, except that an equivalent amount of 1-(2-fluorophenyl)-2-pentanone (prepared as described in Preparation 5) was used in place of the 1-(2-fluorophenyl)-2-butanone, the title compound was obtained as a pale yellow oil in a yield of 41%.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 0.82 (3H, triplet, $J=9.5$ Hz); 1.45-1.70 (2H, multiplet); 2.41 (2H, triplet, $J=8.0$ Hz); 2.75-2.95 (4H, multiplet); 3.55 (1H, doublet, $J=13.0$ Hz); 3.62 (1H, doublet, $J=13.0$ Hz); 4.75 (1H, singlet); 6.65 (1H, doublet, $J=6.0$ Hz); 7.05 (1H, doublet, $J=6.0$ Hz); 7.10-7.55 (4H, multiplet).

Mass spectrum (CI, m/z): 318 ($M^+ + 1$), 246.

13(b) A procedure similar to that described in Example 2(b) was repeated, using the title compound prepared as described in step (a) above, except that maleic acid was added in place of blowing hydrogen chloride gas through the mixture, to obtain the maleate of the title compound as a colorless powder, melting at 89°-90° C., in a yield of 36%.

Elemental analysis: Calculated for $C_{18}H_{20}FNOS \cdot C_4H_4O_4$: C, 60.96%; H, 5.58%; N, 3.23%; Found: C, 60.69%; H, 5.43%; N, 3.01%.

EXAMPLE 14

5-(2-Fluoro- α -valerylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and its maleate (Compound No. 120)

14(a) Following a procedure similar to that described in Example 6, except that an equivalent amount of 1-(2-fluorophenyl)-2-hexanone (prepared as described in Preparation 6) was used in place of the 1-(2-fluorophenyl)-2-butanone, the title compound was obtained as a pale yellow oil in a yield of 46%.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 0.83 (3H, triplet, $J=8.0$ Hz); 1.12-1.35 (2H, multiplet); 1.40-1.70 (2H, multiplet); 2.45 (2H, triplet, $J=8.2$ Hz); 2.60-2.90 (4H, multiplet); 3.52 (1H, doublet, $J=14.0$ Hz); 3.65 (1H, doublet, $J=14.0$ Hz); 4.75 (1H, singlet); 6.65 (1H, doublet, $J=6.0$ Hz); 7.05 (1H, doublet, $J=6.0$ Hz); 7.10-7.50 (4H, multiplet).

Mass Spectrum (CI, m/z): 332 ($M^+ + 1$), 246.

14(b) A procedure similar to that described in Example (b) was repeated, using the title compound prepared as described in step (a) above, except that maleic acid was added in place of blowing hydrogen chloride gas through the mixture, to obtain the maleate of the title compound as a colorless powder, melting at $92^\circ-93^\circ$ C., in a yield of 26%.

Elemental analysis: Calculated for $C_{19}H_{22}FNOS \cdot C_4H_4O_4$: C, 61.73%; H, 5.86%; N, 3.13%; Found: C, 61.38%; H, 5.88%; N, 2.59%.

EXAMPLE 15

5-(2-Fluoro- α -pivaloylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and its hydrochloride (Compound No. 122)

15(a) Following a procedure similar to that described in Example 6, except that an equivalent amount of 1-(2-fluorophenyl)-3,3-dimethyl-2-butanone (prepared as described in Preparation 7) was used in place of the 1-(2-fluorophenyl)-2-butanone, the title compound was obtained as a pale yellow oil in a yield of 87%.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 1.10 (9H, singlet); 2.74-3.00 (4H, multiplet); 3.55 (1H, doublet, $J=15.0$ Hz); 3.66 (1H, doublet, $J=15.0$ Hz); 5.23 (1H, singlet); 6.63 (1H, doublet, $J=6.0$ Hz); 7.03 (1H, doublet, $J=6.0$ Hz); 7.10-7.55 (4H, multiplet).

Mass spectrum (CI, m/z): 332 ($M^+ + 1$), 246.

15(b) A procedure similar to that described in Example 2(b) was repeated, using the title compound prepared as described in step (a) above, to obtain the hydrochloride of the title compound as a pale yellow powder, melting at $85^\circ-90^\circ$ C., in a yield of 34%.

Elemental analysis: Calculated for $C_{19}H_{22}FNOS \cdot HCl \cdot H_2O$: C, 59.14%; H, 6.23%; N, 3.63%; Found: C, 58.99%; H, 6.57%; N, 3.17%.

EXAMPLE 16

5-[2-Chloro- α -(4-fluorobenzoyl)benzyl]-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and its hydrochloride (Compound No. 149)

16(a) Following a procedure similar to that described in Example 6, except that an equivalent amount of 2-chlorobenzyl 4-fluorophenyl ketone (prepared as described in Preparation 22) was used in place of the 1-(2-

fluorophenyl)-2-butanone, the title compound was obtained as a pale yellow oil in a yield of 58%.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 2.80-3.00 (4H, multiplet); 3.63 (1H, doublet, $J=16.0$ Hz); 3.80 (1H, doublet, $J=16.0$ Hz); 5.80 (1H, singlet); 6.63 (1H, doublet, $J=6.0$ Hz); 7.00-7.60 (6H, multiplet); 7.95-8.15 (2H, multiplet).

Mass spectrum (CI, m/z): 386 ($M^+ + 1$), 262.

16(b) A procedure similar to that described in Example 2(b) was repeated, using the title compound prepared as described in step (a) above, to obtain the hydrochloride of the title compound as a yellowish brown powder, melting at $121^\circ-130^\circ$ C., in a yield of 40%.

Elemental analysis: Calculated for $C_{21}H_{17}ClFNOS \cdot HCl \cdot \frac{1}{2}H_2O$: C, 58.47%; H, 4.44%; N, 3.25%; Found: C, 58.25%; H, 4.86%; N, 3.48%.

EXAMPLE 17

5-(2-Fluoro- α -isobutyrylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and its maleate (Compound No. 118)

17(a) Following a procedure similar to that described in Example 6, except that an equivalent amount of 2-fluorobenzyl isopropyl ketone (prepared as described in Preparation 23) was used in place of the 1-(2-fluorophenyl)-2-butanone, the title compound was obtained as a yellow oil in a yield of 44%.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 0.95 (3H, doublet, $J=7.0$ Hz); 1.10 (3H, doublet, $J=7.0$ Hz); 2.60-2.80 (1H, multiplet); 2.80-2.95 (4H, multiplet); 3.50 (1H, doublet, $J=11.0$ Hz); 3.65 (1H, doublet, $J=11.0$ Hz); 4.90 (1H, singlet); 6.65 (1H, doublet, $J=5.7$ Hz); 7.05 (1H, doublet, $J=5.7$ Hz); 7.10-7.50 (4H, multiplet).

Mass spectrum (CI, m/z): 318 ($M^+ + 1$), 246.

17(b) A procedure similar to that described in Example 2(b) was repeated, using the title compound prepared as described in step (a) above, except that maleic acid was added in place of blowing hydrogen chloride gas through the mixture, to obtain the maleate of the title compound as a colorless powder, melting at $96^\circ-98^\circ$ C., in a yield of 42%.

Elemental analysis: Calculated for $C_{18}H_{20}FNOS \cdot C_4H_4O_4$: C, 61.02%; H, 5.59%; N, 3.23%; Found: C, 60.74%; H, 5.52%; N, 3.23%.

EXAMPLE 18

5(α -Cyclopropylcarbonyl-2-fluorobenzyl)-2-nitro-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and its hydrochloride (Compound No. 175)

18(a) Following a procedure similar to that described in Example 6, except that an equivalent amount of cyclopropyl 2-fluorobenzyl ketone (prepared as described in Preparation 8) was used in place of the 1-(2-fluorophenyl)-2-butanone and that 2-nitro-4,5,6,7-tetrahydrothieno[3,2-c]pyridine hydrochloride (prepared as described in Preparation 24) was used in place of the 4,5,6,7-tetrahydrothieno[3,2-c]pyridine hydrochloride, the title compound was obtained as a brown oil in a yield of 72%.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 0.82-0.92 (2H, multiplet); 1.01-1.11 (2H, multiplet); 2.00-2.20 (1H, multiplet); 2.75-3.05 (4H, multiplet); 3.61 (2H, singlet); 4.91 (1H, singlet); 7.10-7.45 (4H, multiplet); 7.55 (1H, singlet).

Mass spectrum (CI, m/z): 361 ($M^+ + 1$), 291.

18(b) A procedure similar to that described in Example 2(b) was repeated, using the title compound prepared as described in step (a) above, to obtain the hydrochloride of the title compound as white crystals, melting at 161°–168° C., in a yield of 79%.

Elemental analysis: Calculated for $C_{18}H_{17}FN_2O_3S \cdot HCl$: C, 54.47%; H, 4.57%; N, 7.06%. Found: C, 54.47%; H, 4.63%; N, 6.89%.

EXAMPLE 19

5-(α -Cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrofuro[3,2-c]pyridine and its hydrochloride (Compound No. 168)

19(a) Following a procedure similar to that described in Example 12, except that an equivalent amount of 4,5,6,7-tetrahydrofuro[3,2-c]pyridine (prepared as described in Preparation 25) was used in place of the 4,5,6,7-tetrahydrothieno[3,2-c]pyridine hydrochloride, the title compound was obtained as a brown oil in a yield of 21%.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 0.75–0.95 (2H, multiplet); 0.98–1.10 (2H, multiplet); 2.15–2.31 (1H, multiplet); 2.65–3.05 (4H, multiplet); 3.40–3.60 (2H, multiplet); 4.90 (1H, singlet); 6.15 (1H, doublet, $J=5.0$ Hz); 7.05–7.55 (5H, multiplet).

Mass spectrum (CI, m/z): 300 ($M^+ + 1$), 230.

19(b) A procedure similar to that described in Example 2(b) was repeated, using the title compound prepared as described in step (a) above, to obtain the hydrochloride of the title compound as white crystals, melting at 154°–155° C., in a yield of 39%.

Elemental analysis: Calculated for $C_{18}H_{18}FNO_2 \cdot HCl$: C, 64.38%; H, 5.70%; N, 4.17%. Found: C, 64.37%; H, 5.80%; N, 4.19%.

EXAMPLE 20

5-(α -Cyclopropylcarbonyl-2-fluorobenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine and its hydrochloride (Compound No. 235)

20(a) Following a procedure similar to that described in Example 12, except that an equivalent amount of 2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine hydrochloride was used in place of the 4,5,6,7-tetrahydrothieno[3,2-c]pyridine hydrochloride, the title compound was obtained as a brown oil in a yield of 32%. Diisopropyl ether was added to this compound to cause crystallization, yielding white crystals, melting at 123°–125° C.

The resulting 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine (Compound No. 235) is believed to contain a small quantity of the tautomeric 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-hydroxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (Compound No. 188), from which it was not separated.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 0.75–0.96 (2H, multiplet); 0.99–1.14 (2H, multiplet); 1.83–2.01 (1H, multiplet); 2.02–2.17 (1H, multiplet); 2.25–2.45 & 2.47–2.62 (together 2H, each multiplet); 2.85 & 3.10 (together 2H, each doublet, $J=12.0$ Hz); 3.88–4.01 & 4.03–4.16 (together 2H, each multiplet); 4.85 & 4.89 (together 1H, each singlet); 6.03 & 6.06 (together 1H, each singlet); 7.10–7.45 (4H, multiplet).

Mass spectrum (CI, m/z): 332 ($M^+ + 1$), 262.

Elemental analysis: Calculated for $C_{18}H_{18}FNO_2S$: C, 65.23%; H, 5.48%; N, 4.23%; Found: C, 65.09%; H, 5.55%; N, 4.20%.

20 (b) A procedure similar to that described in Example 2(b) was repeated, using the title compound prepared as described in step (a) above, to obtain the hydrochloride of the title compound as white crystals, melting at 104°–109° C., in a yield of 46%.

EXAMPLE 21

5-(2-Fluoro- α -propionylbenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine and its hydrochloride (Compound No. 234)

21(a) Following a procedure similar to that described in Example 20, except that an equivalent amount of 1-(2-fluorophenyl)-2-butanone (prepared as described in Preparation 9) was used in place of the cyclopropyl 2-fluorobenzyl ketone, the title compound was obtained as a brown oil in a yield of 36%.

The resulting 5-(2-fluoro- α -propionylbenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine (Compound No. 234) is believed to contain a small quantity of the tautomeric 5-(2-fluoro- α -propionylbenzyl)-2-hydroxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (Compound No. 187).

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 1.00 (3H, triplet, $J=9.1$ Hz); 1.82–1.98 (1H, multiplet); 2.25–2.50 (4H, multiplet); 2.85 & 3.05 (together 2H, each doublet, $J=14.0$ Hz); 3.84–3.95 & 4.04–4.17 (together 2H, each multiplet); 4.72 & 4.76 (together 1H, each singlet); 6.03 & 6.07 (together 1H, each singlet); 7.15–7.40 (4H, multiplet).

Mass spectrum (CI, m/z): 320 ($M^+ + 1$), 262.

21(b) A procedure similar to that described in Example 2(b) was repeated, using the title compound prepared as described in step (a) above, to obtain the hydrochloride of the title compound as white crystals, melting at 110°–115° C. in a yield of 78%.

EXAMPLE 22

5-(2-Chloro- α -cyclopropylcarbonylbenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine (Compound No. 233)

Following a procedure similar to that described in Example 5, except that an equivalent amount of 2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine hydrochloride was used in place of the 4,5,6,7-tetrahydrothieno[3,2-c]pyridine hydrochloride, a yellow oil was obtained. The oil was crystallized from diisopropyl ether to give the title compound as pale brown crystals, melting at 119°–123° C. in a yield of 8%.

The resulting 5-(2-chloro- α -cyclopropylcarbonylbenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine (Compound No. 233) is believed to contain a small quantity of the tautomeric 5-(2-chloro- α -cyclopropylcarbonylbenzyl)-2-hydroxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (Compound No. 186).

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 0.75–1.10 (4H, multiplet); 1.75–2.10 (2H, multiplet); 2.25–2.70 (2H, multiplet); 2.90–3.30 (2H, multiplet); 3.75–4.20 (2H, multiplet); 5.09 & 5.10 (together 1H, each singlet); 5.98 & 6.07 (together 1H, each singlet); 7.10–7.50 (4H, multiplet).

Mass spectrum (CI, m/z): 348 ($M^+ + 1$), 278.

EXAMPLE 23

2-Acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (Compound No. 190)

2.6 g (7.8 mmole) of 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine pyridine (prepared as described in Example 20) were dissolved in a mixture of 10 ml of dimethylformamide and 5 ml of acetic anhydride, and then 0.35 g (8.6 mmole) of a 60% w/w dispersion of sodium hydride in mineral oil was added to the resulting solution, whilst ice-cooling; the mixture was then stirred for 20 minutes at the same temperature, after which it was stirred for a further 3 hours at room temperature. At the end of this time, 300 ml of ethyl acetate were added to the mixture, which was then washed four times, each time with 50 ml of a saturated aqueous solution of sodium chloride. The organic layer was separated and dried over anhydrous sodium sulfate, and the solvent was removed by evaporation under reduced pressure. The resulting residue was subjected to silica gel column chromatography, using a 100:3 by volume mixture of toluene and ethyl acetate as the eluent, to give a yellow oil. This oil was crystallized from diisopropyl ether, to obtain the title compound as white crystals, melting at 120°–121.5° C., in a yield of 65%.

Infrared Absorption Spectrum (KBr) ν_{\max} cm⁻¹: 1758, 1704.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 0.80–0.95 (2H, multiplet); 0.99–1.16 (2H, multiplet); 2.27 (3H, singlet); 2.21–2.34 (1H, multiplet); 2.70–2.95 (4H, multiplet); 3.47 (1H, doublet, $J=15.0$ Hz); 3.57 (1H, doublet, $J=15.0$ Hz); 4.83 (1H, singlet); 6.27 (1H, singlet); 7.10–7.55 (4H, multiplet).

Mass spectrum (CI, m/z): 374 ($M^+ + 1$), 304.

Elemental analysis: Calculated for C₂₀H₂₀FNO₃S: C, 64.32%; H, 5.40%; N, 3.75%; Found: C, 64.46%; H, 5.39%; N, 3.73%.

EXAMPLE 24

5-(α -Cyclopropylcarbonyl-2-fluorobenzyl)-2-propionyloxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (Compound No. 192)

Following a procedure similar to that described in Example 23, except that an equivalent amount of propionic anhydride was used in place of the acetic anhydride, the title compound was obtained as white crystals, melting at 101°–102° C., in a yield of 16%.

Infrared Absorption Spectrum (KBr) ν_{\max} cm⁻¹: 1705, 1760.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 0.75–0.90 (2H, multiplet); 0.90–1.10 (2H, multiplet); 1.21 (3H, triplet, $J=6.7$ Hz); 2.15–2.37 (1H, multiplet); 2.55 (2H, quartet, $J=6.7$ Hz); 2.65–2.95 (4H, multiplet); 3.40–3.60 (2H, multiplet); 4.80 (1H, singlet); 6.25 (1H, singlet); 7.05–7.55 (4H, multiplet).

Mass spectrum (CI, m/z): 388 ($M^+ + 1$), 318.

Elemental analysis: Calculated for C₂₁H₂₂FNO₃S: C, 65.10%; H, 5.72%; N, 3.61%; Found: C, 64.80%; H, 5.72%; N, 3.61%.

EXAMPLE 25

2-Butyryloxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (Compound No. 194)

Following a procedure similar to that described in Example 23, except that an equivalent amount of butyric anhydride was used in place of the acetic anhydride, the title compound was obtained as white crystals, melting at 84°–85° C., in a yield of 39%.

Infrared Absorption Spectrum (KBr) ν_{\max} cm⁻¹: 1756, 1706.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 0.75–1.10 (7H, multiplet); 1.65–1.85 (2H, multiplet); 2.21–2.34 (1H, multiplet); 2.49 (2H, triplet, $J=7.0$ Hz); 2.70–3.00 (4H, multiplet); 3.52 (2H, broad triplet, $J=16.0$ Hz); 4.82 (1H, singlet); 6.25 (1H, singlet); 7.05–7.55 (4H, multiplet).

Mass spectrum (CI, m/z): 402 ($M^+ + 1$), 332.

Elemental analysis: Calculated for C₂₂H₂₄FNO₃S: C, 65.81%; H, 6.03%; N, 3.49%. Found: C, 65.92%; H, 5.91%; N, 3.41%.

EXAMPLE 26

5-(α -Cyclopropylcarbonyl-2-fluorobenzyl)-2-pivaloyloxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (Compound No. 196)

Following a procedure similar to that described in Example 23, except that an equivalent amount of pivalic anhydride was used in place of the acetic anhydride, the title compound was obtained as white crystals, melting at 91°–94° C., in a yield of 44%.

Infrared Absorption Spectrum (KBr) ν_{\max} cm⁻¹: 1749, 1700.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 0.79–0.92 (2H, multiplet); 0.98–1.09 (2H, multiplet); 1.31 (9H, singlet); 2.23–2.36 (1H, multiplet); 2.70–2.95 (4H, multiplet); 3.47 (1H, doublet, $J=14.5$ Hz); 3.58 (1H, doublet, $J=14.5$ Hz); 4.83 (1H, singlet); 6.26 (1H, singlet); 7.05–7.55 (4H, multiplet).

Mass spectrum (CI, m/z): 416 ($M^+ + 1$), 346.

Elemental analysis: Calculated for C₂₃H₂₆FNO₃S: C, 66.48%; H, 6.31%; N, 3.37%; Found: C, 66.21%; H, 6.40%; N, 3.38%.

EXAMPLE 27

5-(α -Cyclopropylcarbonyl-2-fluorobenzyl)-2-nonanoyloxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (Compound No. 199)

1.0 g (3.0 mmole) of 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine (prepared as described in Example 20) was dissolved in 15 ml of dimethylformamide, and then 0.18 g (4.5 mmole) of a 60% w/w dispersion of sodium hydride in mineral oil and 0.82 ml (4.5 mmole) of nonanoyl chloride were added, in that order, to the resulting mixture, whilst ice-cooling. The resulting reaction mixture was then stirred at the same temperature for 30 minutes, after which it was stirred at room temperature for a further 5 hours. 300 ml of ethyl acetate were then added to the mixture, which was then washed with a saturated aqueous solution of sodium hydrogencarbonate and with a saturated aqueous solution of sodium chloride, in that order. The organic layer was separated and dried over anhydrous sodium sulfate, and the solvent was removed by evaporation under reduced pres-

sure. The resulting residue was subjected to silica gel column chromatography, using a 100:2 by volume mixture of toluene and ethyl acetate as the eluent, to give a yellow oil. The oil was crystallized from petroleum ether to obtain the title compound as white crystals, melting at 45°–48° C., in a yield of 40%.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 0.80–1.80 (19H, multiplet); 2.21–2.32 (1H, multiplet); 2.53 (2H, triplet, $J=7.5$ Hz); 2.70–2.95 (4H, multiplet); 3.48 (1H, doublet, $J=15.0$ Hz); 3.57 (1H, doublet, $J=15.0$ Hz); 4.84 (1H, singlet); 6.27 (1H, singlet); 7.05–7.55 (4H, multiplet).

Mass spectrum (CI, m/z): 472 ($M^+ + 1$), 402.

Elemental analysis: Calculated for C₂₇H₃₄FNO₃S: C, 68.76%; H, 7.27%; N, 2.97%. Found: C, 68.56%; H, 7.49%; N, 2.97%.

EXAMPLE 28

5-(α -Cyclopropylcarbonyl-2-fluorobenzyl)-2-decanoyloxy-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine and its hydrochloride (Compound No. 200)

28(a) Following a procedure similar to that described in Example 27, except that an equivalent amount of decanoyl chloride was used in place of the nonanoyl chloride, the title compound was obtained as a yellow oil in a yield of 40%.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 0.80–1.80 (21H, multiplet); 2.18–2.32 (1H, multiplet); 2.52 (2H, triplet, $J=7.5$ Hz); 2.70–2.97 (4H, multiplet); 3.50 (1H, doublet, $J=14.5$ Hz); 3.59 (1H, doublet, $J=14.5$ Hz); 4.85 (1H, singlet); 6.26 (1H, singlet); 7.20–7.55 (4H, multiplet).

Mass spectrum (CI, m/z): 486 ($M^+ + 1$), 416.

28(b) A procedure similar to that described in Example 2(b) was repeated, using the title compound prepared as described in step (a) above, except that diisopropyl ether was used as a solvent in place of the diethyl ether, to give the hydrochloride of the title compound as yellow crystals, melting at 62°–64° C., in a yield of 81%.

Elemental analysis: Calculated for C₂₈H₃₆FNO₃S.HCl: C, 64.41%; H, 7.14%; N, 2.68%. Found: C, 64.12%; H, 7.05%; N, 2.63%.

EXAMPLE 29

5-(α -Cyclopropylcarbonyl-2-fluorobenzyl)-2-palmitoyloxy-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (Compound No. 201)

Following a procedure similar to that described in Example 27, except that an equivalent amount of palmitoyl chloride was used in place of the nonanoyl chloride, the title compound was obtained as white crystals, melting at 6620–68° C., in a yield of 21%.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 0.80–1.80 (33H, multiplet); 2.20–2.32 (1H, multiplet); 2.51 (2H, triplet, $J=7.5$ Hz); 2.70–2.95 (4H, multiplet); 3.48 (1H, doublet, $J=15.0$ Hz); 3.58 (1H, doublet, $J=15.0$ Hz); 4.84 (1H, singlet); 6.26 (1H, singlet); 7.10–7.55 (4H, multiplet).

Mass spectrum (CI, m/z): 570 ($M^+ + 1$), 500.

Elemental analysis: Calculated for C₃₄H₄₈FNO₃S: C, 71.66%; H, 8.49%; N, 2.46%. Found: C, 71.72%; H, 8.62%; N, 2.43%.

EXAMPLE 30

2-t-Butoxycarbonyloxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (Compound No. 203)

Following a procedure similar to that described in Example 23, except that an equivalent amount of di-*t*-butyl dicarbonate was used in place of the acetic anhydride, the title compound was obtained as white crystals, melting at 98°–99° C., in a yield of 15%.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 0.80–0.90 (2H, multiplet); 0.98–1.09 (2H, multiplet); 1.55 (9H, singlet); 2.20–2.34 (1H, multiplet); 2.70–2.95 (4H, multiplet); 3.40–3.60 (2H, multiplet); 4.83 (1H, singlet); 6.27 (1H, singlet); 7.07–7.52 (4H, multiplet).

Mass spectrum (CI, m/z): 432 ($M^+ + 1$), 362.

Elemental analysis: Calculated for C₂₃H₂₆FNO₄S: C, 64.02%; H, 6.07%; N, 3.25%. Found: C, 63.57%; H, 6.03%; N, 3.27%.

EXAMPLE 31

2-Amino-5-(α -Cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (Compound No. 177)

5 ml of hydrochloric acid were added to 0.4 g of 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-nitro-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine hydrochloride (prepared as described in Example 18), and then 0.23 g of tin powder was added to the resulting mixture, whilst stirring, after which the mixture was stirred at room temperature for a further hour. 10 ml of water were added to the reaction mixture, which was then extracted with methylene chloride. The methylene chloride layer was removed, and the aqueous layer was concentrated to dryness by evaporation under reduced pressure, and then crystallized from diethyl ether, to give a complex of the title compound with stannic chloride as a pale yellow powder in a yield of 72%.

Nuclear Magnetic Resonance Spectrum (CD₃OD) δ ppm: 0.95–1.05 (2H, multiplet); 1.20–1.35 (2H, multiplet); 1.85–1.99 (1H, multiplet); 3.60–3.80 (2H, multiplet); 6.07 (1H, singlet); 7.35–7.80 (4H, multiplet).

EXAMPLE 32

2-Acetyl-amino-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (Compound No. 179)

1.85 g (5.13 mmole) of 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-nitro-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine (prepared as described in Example 18) were dissolved in a mixture of 20 ml of acetic acid and 2 ml of acetic anhydride, and then 1.85 g of iron powder were added to the solution, whilst stirring at room temperature; the mixture was then stirred at the same temperature for 90 minutes. At the end of this time, water and chloroform were added to the reaction mixture, and the mixture was neutralized with sodium carbonate. The inorganic salt thus precipitated was filtered off, the remaining organic layer was separated and the aqueous layer was extracted with chloroform. The organic layer and the extract were combined and dried over anhydrous magnesium sulfate, and then the solvent was removed by distillation under reduced pressure. The resulting residue was then subjected to silica gel column chromatography, using a 6:4 by volume mixture of

toluene and ethyl acetate as the eluent, to give 1.86 g of the title compound. This was crystallized from diisopropyl ether to obtain 1.37 g of the title compound as white crystals, melting at 155°–159° C.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 0.78–0.94 (2H, multiplet); 0.98–1.12 (2H, multiplet); 2.17 (3H, singlet); 2.15–2.32 (1H, multiplet); 2.70–2.99 (4H, multiplet); 3.50 (1H, doublet, $J=11.4$ Hz); 3.60 (1H, doublet, $J=11.4$ Hz); 4.86 (1H, singlet); 6.27 (1H, singlet); 7.10–7.55 (4H, multiplet); 7.80–8.00 (1H, broad singlet).

Mass spectrum (CI, m/z): 373 ($M^+ + 1$), 303.

Elemental analysis: Calculated for C₂₀H₂₁FN₂O₂S: C, 64.49%; H, 5.68%; N, 7.52%; Found: C, 64.38%; H, 5.50%; N, 7.38%.

EXAMPLE 33

2-Butyrylamino-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (Compound No. 181)

Following a procedure similar to that described in Example 32, except that equivalent amounts of butyric acid and butyric anhydride were used in place of the acetic acid and acetic anhydride, the title compound was obtained as white crystals, melting at 154°–157° C., in a yield of 61%.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 0.78–0.94 (2H, multiplet); 0.90–1.10 (5H, multiplet); 1.65–1.82 (2H, multiplet); 2.21–2.39 (3H, multiplet); 2.69–2.95 (4H, multiplet); 3.47 (1H, doublet, $J=11.4$ Hz); 3.56 (1H, doublet, $J=11.4$ Hz); 4.81 (1H, singlet); 6.25 (1H, singlet); 7.10–7.60 (4H, multiplet); 7.70 (1H, singlet).

Mass spectrum (CI, m/z): 401 ($M^+ + 1$), 331.

Elemental analysis: Calculated for C₂₂H₂₅FN₂O₂: C, 65.97%; H, 6.29%; N, 6.99%; Found: C, 65.95%; H, 6.36%; N, 6.95%.

EXAMPLE 34

Optically active

5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (Compound No. 59)

0.3 g of 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (prepared as described in Example 12) was separated into fractions by liquid chromatography [column: DAICEL CHIRALPAC AD (trade name), 1 cm \times 25 cm; eluent: a 1000:40:1 by volume mixture of hexane, isopropanol and diethylamine; column temperature: 35° C.; flow rate: 4 ml/minute], to obtain an optically active isomer A [retention time: 8.3 minutes; specific rotation angle $[\alpha]_D^{25}$: 109.4° ($C=1.80$, CHCl₃)] and an isomer B [retention time: 9.9 minutes; specific rotation angle $[\alpha]_D^{25}$: 100.1° ($C=1.90$, CHCl₃)].

Isomers A and B were separately dissolved in diethyl ether, and then hydrogen chloride gas was allowed to act upon the resulting solutions to obtain 0.13 g and 0.12 g of the hydrochlorides of isomer A and isomer B, respectively, as white crystals.

Hydrochloride of isomer A

melting at 106°–110° C.

Elemental analysis: Calculated for C₁₈H₁₈FNOS.HCl. $\frac{1}{2}$ H₂O: C, 59.17%; H, 5.65%; N, 3.83%; Found: C, 59.06%; H, 5.74%; N, 3.90%.

Hydrochloride of isomer B

melting at 105°–110° C.

Elemental analysis: Calculated for C₁₈H₁₈FNOS.HCl. $\frac{1}{2}$ H₂O: C, 59.91%; H, 5.59%; N, 3.88%; Found: C, 59.80%; H, 5.84%; N, 3.79%.

EXAMPLE 35

5-(α -Cyclopropylcarbonyl-2-fluorobenzyl)-2-pivaloyloxymethoxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (Compound No. 207)

1.0 g (3.0 mmole) of 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine (prepared as described in Example 20) was dissolved in 20 ml of dimethylformamide, and then 100 mg (0.6 mmole) of potassium iodide and 0.13 g (3.3 mmole) of a 60% dispersion of sodium hydride in mineral oil were added to the solution at room temperature; the mixture was then stirred at the same temperature for 10 minutes. At the end of this time, a solution of 0.43 ml (3.0 mmole) of pivaloyloxymethyl chloride in 5 ml of dimethylformamide was added dropwise to the resulting mixture over a period of 10 minutes, and the resulting mixture was stirred at room temperature for 30 minutes. 300 ml of ethyl acetate were added to the reaction mixture, and the mixture was washed three times, each time with 50 ml of a saturated aqueous solution of sodium hydrogen carbonate. The organic layer was dried over anhydrous sodium sulfate, and the solvent was removed by evaporation under reduced pressure. The resulting residue was subjected to silica gel column chromatography, using a 100:3 by volume mixture of toluene and ethyl acetate as the eluent, to give the title compound as a colorless oil in a yield of 15%.

Infrared Absorption Spectrum (thin film) ν_{max} cm⁻¹: 1715, 1702.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 0.79–0.93 (2H, multiplet); 0.99–1.14 (2H, multiplet); 1.22 (9H, singlet); 2.18–2.31 (1H, multiplet); 2.65–2.95 (4H, multiplet); 3.44 (1H, doublet, $J=15.5$ Hz); 3.55 (1H, doublet, $J=15.5$ Hz); 4.84 (1H, singlet); 5.57 (2H, singlet); 6.04 (1H, singlet); 7.05–7.50 (4H, multiplet).

Mass spectrum (CI, m/z): 446 ($M^+ + 1$), 376.

EXAMPLE 36

5-(α -Cyclopropylcarbonyl-2-fluorobenzyl)-2-methoxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and its hydrochloride (Compound No. 210)

36(a) A procedure similar to that described in Example 35 was repeated, except that an equivalent amount of methyl iodide was used in place of the pivaloyloxymethyl chloride and potassium iodide, to give the title compound as a yellow oil in a yield of 45%.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 0.80–0.92 (2H, multiplet); 1.00–1.10 (2H, multiplet); 2.20–2.36 (1H, multiplet); 2.65–2.96 (4H, multiplet); 3.42 (1H, doublet, $J=14.5$ Hz); 3.55 (1H, doublet, $J=14.5$ Hz); 3.80 (3H, singlet); 4.82 (1H, singlet); 5.80 (1H, singlet); 7.10–7.60 (4H, multiplet).

Mass spectrum (CI, m/z): 346 ($M^+ + 1$), 276.

36(b) Following a procedure similar to that described in Example 2(b), using the whole of the title compound prepared as described in step (a) above, the hydrochloride of the title compound was obtained as white crystals, melting at 102°–106° C., in a yield of 78%.

Elemental analysis:

Calculated for $C_{19}H_{20}FNO_2S \cdot HCl \cdot \frac{1}{2}H_2O$: C, 58.38%; H, 5.67%; N, 3.58%; Found: C, 58.08%; H, 5.77%; N, 3.53%.

EXAMPLE 37

5-[α -(2-Fluorocyclopropylcarbonyl)-2-fluorobenzyl]-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine (Compound No. 275)

Following a procedure similar to that described in Example 1, except that equivalent amounts of 2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine hydrochloride and 2-fluoro- α -(2-fluorocyclopropylcarbonyl)-benzyl bromide (prepared as described in Preparation 27) were used in place of the 4,5,6,7-tetrahydrothieno[3,2-c]pyridine hydrochloride and 2-chloro- α -trifluoroacetylbenzyl bromide, the title compound was obtained as a yellow oil in a yield of 31%.

The resulting 5-[α -(2-fluorocyclopropylcarbonyl)-2-fluorobenzyl]-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine (Compound No. 275) is believed to contain a small quantity of the tautomeric 5-[α -(2-fluorocyclopropylcarbonyl)-2-fluorobenzyl]-2-hydroxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine (Compound No. 274), from which it was not separated.

Infrared Absorption Spectrum (thin film) ν_{max} cm^{-1} : 1680.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 1.48-1.55 (2H, multiplet); 1.85-2.01 (1H, multiplet); 2.30-2.51 (2H, multiplet); 2.53-2.90 (1H, multiplet); 3.00-3.20 (2H, multiplet); 3.83-4.01 & 4.03-4.18 (together 2H, each multiplet); 4.46-4.60 & 4.79-4.92 (together 2H, each multiplet); 6.05 & 6.09 (together 1H, each singlet); 7.10-7.45 (4H, multiplet). Mass spectrum (CI, m/z): 350 ($M+1$), 262.

PREPARATION 1

3-(2-Chlorobenzyl)-5,6-dihydro-1,4,2-dioxazine

A solution of 5.0 g (29.3 mmole) of o-chlorophenylacetic acid and 0.3 g of p-toluenesulfonic acid monohydrate in 50 ml of methanol was heated under reflux for 6 hours. At the end of this time, 3.1 g (44 mmole) of hydroxylamine hydrochloride were added to the reaction mixture, followed by 2.1 g of sodium methoxide. The resulting reaction mixture was then heated under reflux for 10 hours. 14.2 g (103 mmole) of potassium carbonate and 5.1 ml of 1,2-dibromoethane were then added to the resulting reaction mixture, followed by 15 ml of water. The reaction mixture was then heated under reflux for a further 10 hours. At the end of this time, 200 ml of ethyl acetate were added to the reaction mixture, and the organic layer was separated, washed with a saturated aqueous solution of sodium hydrogen carbonate and dried over anhydrous sodium sulfate; the solvent was then removed by distillation under reduced pressure. The residue thus obtained was subjected to silica gel column chromatography, using a 9:1 by volume mixture of toluene and ethyl acetate as the eluent, to give 4.9 g of the title compound as an oil.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 3.67 (2H, singlet); 4.05 (2H, triplet, $J=4.2$ Hz); 4.29 (2H, triplet, $J=4.2$ Hz); 7.10-7.40 (4H, multiplet). Mass spectrum (CI, m/z): 212 ($M+1$), 176.

PREPARATION 2

3-(2-Fluorobenzyl)-5,6-dihydro-1,4,2-dioxazine

A procedure similar to that described in Preparation 1 was repeated, except that an equivalent amount of o-fluorophenylacetic acid was used in place of the o-chlorophenylacetic acid, to give the title compound as a colorless oil in a yield of 45%.

Mass spectrum (CI, m/z): 196 ($M+1$), 109.

PREPARATION 3

3-(2,6-Difluorobenzyl)-5,6-dihydro-1,4,2-dioxazine

A procedure similar to that described in Preparation 1 was repeated, except that an equivalent amount of 2,6-difluorophenylacetic acid was used in place of the o-chlorophenylacetic acid, to give the title compound as a colorless oil in a yield of 45%.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 3.61 (2H, singlet); 4.04 (2H, triplet, $J=4.1$ Hz); 4.30 (2H, triplet, $J=4.1$ Hz); 6.80-7.30 (4H, multiplet).

Mass spectrum (CI, m/z): 214 ($M+1$), 127.

PREPARATION 4

2-Chlorobenzyl cyclopropyl ketone

10 ml of anhydrous diethyl ether were added to 0.45 g (18.5 mmole) of metallic magnesium, and then a solution of 2.0 ml (15.4 mmole) of 2-chlorobenzyl bromide in 10 ml of diethyl ether was slowly added dropwise to the resulting mixture, whilst stirring; the mixture was then stirred at room temperature for one hour. The resulting solution was slowly added dropwise to a solution of 1.1 ml of cyclopropyl cyanide in 10 ml of diethyl ether over a period of 30 minutes, and then the mixture was stirred at room temperature for 2 hours. At the end of this time, a saturated aqueous solution of ammonium chloride was added to the reaction mixture, and the mixture was stirred at room temperature for 15 minutes. 200 ml of ethyl acetate were then added to the reaction mixture, and the organic layer was separated, washed with water, with a saturated aqueous solution of sodium hydrogencarbonate and with a saturated aqueous solution of sodium chloride, in that order, and dried over anhydrous sodium sulfate; the solvent was then removed by distillation under reduced pressure. The residue thus obtained was subjected to silica gel column chromatography, using a 9:1 by volume mixture of toluene and ethyl acetate as the eluent, to give 2.0 g of the title compound as a colorless oil.

Infrared Absorption Spectrum (thin film) ν_{max} cm^{-1} : 1695.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 0.86-0.92 (2H, multiplet); 1.06-1.12 (2H, multiplet); 1.96-2.02 (1H, multiplet); 3.98 (2H, singlet); 7.10-7.50 (4H, multiplet).

Mass spectrum (CI, m/z): 195 ($M+1$), 159.

PREPARATION 5

1-(2-Fluorophenyl)-2-pentanone

A procedure similar to that described in Preparation 4 was repeated, except that equivalent amounts of 2-fluorobenzyl bromide and butyl cyanide were used in place of the 2-chlorobenzyl bromide and cyclopropyl cyanide, to give the title compound as a colorless oil in a yield of 36%.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 0.90 (3H, triplet, $J=8.0$ Hz); 1.52-1.73 (2H, multi-

plet); 2.45 (2H, triplet, $J=8.0$ Hz); 3.70 (2H, singlet); 7.00–7.30 (4H, multiplet).

Mass spectrum (CI, m/z): 181 ($M+1$), 109.

PREPARATION 6

1-(2-Fluorophenyl)-2-hexanone

A procedure similar to that described in Preparation 4 was repeated, except that equivalent amounts of 2-fluorobenzyl bromide and pentyl cyanide were used in place of the 2-chlorobenzyl bromide and cyclopropyl cyanide, to give the title compound as a colorless oil in a yield of 46%.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 0.90 (3H, triplet, $J=8.0$ Hz); 1.20–1.39 (2H, multiplet); 1.50–1.65 (2H, multiplet); 2.50 (2H, triplet, $J=8.0$ Hz); 3.70 (2H, singlet); 7.00–7.30 (4H, multiplet).

Mass spectrum (CI, m/z): 195 ($M+1$), 109.

PREPARATION 7

1-(2-Fluorophenyl)-3,3-dimethyl-2-butanone

A procedure similar to that described in Preparation 4 was repeated, except that equivalent amounts of 2-fluorobenzyl bromide and *t*-butyl cyanide were used in place of the 2-chlorobenzyl bromide and cyclopropyl cyanide, to give the title compound as a colorless oil in a yield of 42%.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 1.25 (9H, singlet); 3.80 (2H, singlet); 7.00–7.30 (4H, multiplet).

Mass spectrum (CI, m/z): 195 ($M+1$), 109.

PREPARATION 8

Cyclopropyl 2-fluorobenzyl ketone

A procedure similar to that described in Preparation 4 was repeated, except that equivalent amounts of 2-fluorobenzyl bromide and cyclopropyl cyanide were used in place of the 2-chlorobenzyl bromide and cyclopropyl cyanide, to give the title compound as a colorless oil in a yield of 70%.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 0.82–0.98 (2H, multiplet); 1.03–1.17 (2H, multiplet); 1.92–2.06 (1H, multiplet); 3.86 (2H, singlet); 7.10–7.30 (4H, multiplet).

Mass spectrum (CI, m/z): 179 ($M+1$).

PREPARATION 9

1-(2-Fluorophenyl)-2-butanone

(a) 1-(2-Fluorophenyl)-2-nitro-1-butene 30 ml of acetic acid were added to 4.73 g (38.11 mmole) of 2-fluorobenzaldehyde, 4.41 g (49.49 mmole) of nitropropane and 3.23 g (41.90 mmole) of ammonium acetate, and the resulting mixture was heated under reflux, whilst stirring, for 4 hours. At the end of this time, the reaction mixture was cooled to room temperature, neutralized with an aqueous solution of sodium hydrogen-carbonate and extracted with diethyl ether. The extract was dried over anhydrous magnesium sulfate, and then xylene was added to the solution. The mixture was concentrated by evaporation under reduced pressure, to give 7.4 g of the title compound as a pale yellow oil.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 1.25 (3H, triplet, $J=6.5$ Hz); 2.80 (2H, quartet, $J=6.5$ Hz); 7.00–7.60 (4H, multiplet); 8.03 (1H, singlet).

Mass spectrum (CI, m/z): 196 ($M+1$), 149.

9(b) 1-(2-Fluorophenyl)-2-butanone

100 ml of 90% v/v aqueous acetic acid were added to 7.4 g of 1-(2-fluorophenyl)-2-nitro-1-butene [prepared

as described in step (a) above], and then 12.11 g (190 mmole) of a zinc powder were added in portions to the resulting solution, whilst heating. The mixture was then heated under reflux, whilst stirring, for 4 hours. At the end of this time, the reaction mixture was left to stand overnight, and then the crystals which had precipitated were filtered off and washed with toluene. The filtrate was combined with the toluene washings, and the mixture was concentrated by evaporation under reduced pressure. The residue thus obtained was subjected to silica gel column chromatography, using toluene as the eluent, to give 1.85 g of the title compound as a pale brown oil.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 1.05 (3H, triplet, $J=7.0$ Hz); 2.53 (2H, quartet, $J=7.0$ Hz); 3.73 (2H, singlet); 7.00–7.40 (4H, multiplet).

Mass spectrum (CI, m/z): 167 ($M+1$), 109.

PREPARATION 10

1-(2-Chlorophenyl)-2-propanone

Following a procedure similar to that described in Preparation 9, except that equivalent amounts of 2-chlorobenzaldehyde and nitroethane were used in place of the 2-fluorobenzaldehyde and nitropropane, the title compound was obtained as a brown oil in a yield of 27%.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 2.20 (3H, singlet); 3.85 (2H, singlet); 7.15–7.45 (4H, multiplet).

Mass spectrum (CI, m/z): 169 ($M+1$), 125.

PREPARATION 11

1-(2-chlorophenyl)-2-butanone

Following a procedure similar to that described in Preparation 9, except that an equivalent amount of 2-chlorobenzaldehyde was used in place of the 2-fluorobenzaldehyde, the title compound was obtained as a pale yellow oil in a yield of 17%.

Mass spectrum (CI, m/z): 183 ($M+1$), 125.

PREPARATION 12

1-(2-Chlorophenyl)-2-heptanone

Following a procedure similar to that described in Preparation 9, except that equivalent amounts of 2-chlorobenzaldehyde and nitrohexane were used in place of the 2-fluorobenzaldehyde and nitropropane, the title compound was obtained as a pale yellow oil in a yield of 17%.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 0.90 (3H, triplet, $J=8.0$ Hz); 1.20–1.40 (4H, multiplet); 1.50–1.70 (2H, multiplet); 2.50 (2H, triplet, $J=10.0$ Hz); 3.80 (2H, singlet); 7.20–7.60 (4H, multiplet).

Mass spectrum (CI, m/z): 225 ($M+1$), 125.

PREPARATION 13

Cyclobutyl 2-fluorobenzyl ketone

20 ml of anhydrous diethyl ether were added to 1.06 g (44 mmole) of metallic magnesium, and then a solution of 7.56 g (40 mmole) of 2-fluorobenzyl bromide in 10 ml of diethyl ether was slowly added dropwise to the resulting mixture, whilst stirring; the mixture was then stirred at room temperature for 1 hour. The resulting solution was slowly added dropwise to a solution of 4.74 g (40 mmole) of cyclobutanecarbonyl chloride in

30 ml of tetrahydrofuran, whilst cooling in a methanol-dry ice bath, over a period of 2 hours, and then the mixture was allowed to return to room temperature, whilst stirring, over a period of 2 hours. At the end of this time, 100 ml of water and 150 ml of diethyl ether were added to the reaction mixture, and the organic layer was separated, dried over anhydrous magnesium sulfate and concentrated by evaporation under reduced pressure. The residue thus obtained was subjected to silica gel column chromatography, using a 9:1 by volume mixture of toluene and hexane as the eluent, to give 2.97 g of the title compound as a pale yellow oil.

Nuclear Magnetic Resonance Spectrum (CDCl_3) δ ppm: 1.65-2.40 (6H, multiplet); 3.31-3.48 (1H, multiplet); 3.67 (2H, singlet); 7.00-7.30 (4H, multiplet).

Mass spectrum (CI, m/z): 193 ($M^+ + 1$), 137.

PREPARATION 14

5-Chloro-1-(2-chlorophenyl)-2-pentanone

Following a procedure similar to that described in Preparation 13, except that equivalent amounts of 2-chlorobenzyl bromide and 4-chlorobutyryl chloride were used in place of the 2-fluorobenzyl bromide and cyclobutanecarbonyl chloride, the title compound was obtained as a yellow oil in a yield of 79%.

Nuclear Magnetic Resonance Spectrum (CDCl_3) δ ppm: 1.96-2.15 (2H, multiplet); 2.69 (2H, triplet, $J=7.7$ Hz); 3.56 (2H, triplet, $J=7.7$ Hz); 3.86 (2H, singlet); 7.10-7.50 (4H, multiplet).

PREPARATION 15

1-(2-Chlorophenyl)-3,3,3-trifluoro-2-propanone

10 ml of anhydrous diethyl ether were added to 0.9 g (37.0 mmole) of metallic magnesium, and then a solution of 3.9 ml (30.8 mmole) of 2-chlorobenzyl chloride in 10 ml of diethyl ether was slowly added dropwise to the resulting mixture, with vigorous stirring, over a period of 30 minutes; the mixture was then stirred at room temperature for 1 hour. The resulting solution was slowly added dropwise to a solution of 4.3 ml (30.8 mmole) of trifluoroacetic anhydride in 40 ml of tetrahydrofuran, whilst cooling to about -70°C ., and then the mixture was allowed to return to room temperature, whilst stirring, over a period of about 1 hour; after this, the mixture was left to stand overnight. At the end of this time, 200 ml of ethyl acetate were added to the resulting reaction mixture, and the organic layer was separated, washed with 1N aqueous hydrochloric acid and with a saturated aqueous solution of sodium chloride, in that order, dried over anhydrous sodium sulfate and concentrated by evaporation under reduced pressure. The residue thus obtained was subjected to silica gel column chromatography, using a 10:2 by volume mixture of toluene and ethyl acetate as the eluent, to give 5.7 g of the title compound as a yellow oil.

Nuclear Magnetic Resonance Spectrum (CDCl_3) δ ppm: 4.16 (2H, singlet); 7.10-7.50 (4H, multiplet).

Mass spectrum (CI, m/z): 223 ($M^+ + 1$), 125.

PREPARATION 16

2-Chloro- α -trifluoroacetylbenzyl bromide

2.0 g (9.0 mmole) of 1-(2-chlorophenyl)-3,3,3-trifluoro-2-propanone were dissolved in 30 ml of carbon tetrachloride, and then 0.46 ml (9.0 mmole) of bromine was added to the solution, which was then stirred at room temperature for 10 hours. At the end of this time, sodium hydrogensulfite was added to the reaction mixture, and the mixture was stirred at room temperature for 15 minutes, after which insolubles were removed by filtration. The filtrate was concentrated by evaporation under reduced pressure, and the residue was subjected to silica gel column chromatography, using a 10:2 by volume mixture of toluene and ethyl acetate as the eluent, to give 0.87 g of the title compound as a yellow oil.

Nuclear Magnetic Resonance Spectrum (CDCl_3) δ ppm: 6.39 (1H, singlet); 7.30-7.70 (4H, multiplet).

Mass spectrum (CI, m/z): 302 ($M^+ + 2$), 300 (M^+), 221.

PREPARATION 17

2-Chloro- α -(4-chlorobutyryl)benzyl bromide

Following a procedure similar to that described in Preparation 16, except that an equivalent amount of 1-(2-chlorophenyl)-5-chloro-2-pentanone was used in place of the 1-(2-chlorophenyl)-3,3,3-trifluoro-2-propanone, the title compound was obtained as a yellow oil in a yield of 72%.

Nuclear Magnetic Resonance Spectrum (CDCl_3) δ ppm: 2.01-2.14 (2H, multiplet); 2.40-2.90 (2H, multiplet); 3.49-3.61 (2H, multiplet); 5.98 (1H, singlet); 7.20-7.60 (4H, multiplet).

Mass spectrum (CI, m/z): 311 ($M^+ + 1$), 231.

PREPARATION 18

2-Chloro- α -(5,6-dihydro-1,4,2-dioxazin-3-yl)benzyl bromide

4.0 g (19 mmole) of 3-(2-chlorobenzyl)-5,6-dihydro-1,4,2-dioxazine (prepared as described in Preparation 1) were dissolved in 40 ml of carbon tetrachloride, and then 4.1 g (23 mmole) of *N*-bromosuccinimide and 0.2 g of benzoyl peroxide were added to the solution, which was then stirred, whilst heating, for 8 hours. At the end of this time, 100 ml of ethyl acetate and 100 ml of hexane were added to the solution, and the mixture was stirred at room temperature for 30 minutes; insolubles were then removed by filtration. The filtrate was concentrated by evaporation under reduced pressure, to give 4.8 g of the title compound as a yellow oil.

Mass spectrum (CI, m/z): 292 ($M^+ + 3$), 290 ($M^+ + 1$), 212.

PREPARATION 19

2-Fluoro- α -(5,6-dihydro-1,4,2-dioxazin-3-yl)benzyl bromide

Following a procedure similar to that described in Preparation 18, except that an equivalent amount of 3-(2-fluorobenzyl)-5,6-dihydro-1,4,2-dioxazine (prepared as described in Preparation 2) was used in place of the 3-(2-chlorobenzyl)-5,6-dihydro-1,4,2-dioxazine, the title compound was obtained as a red oil in a yield of 98%.

Mass spectrum (CI, m/z): 276 ($M^+ + 3$), 194.

PREPARATION 20

2,6-Difluoro- α -(5,6-dihydro-1,4,2-dioxazin-3-yl)benzyl bromide

Following a procedure similar to that described in Preparation 18, except that an equivalent amount of 3-(2,6-difluorobenzyl)-5,6-dihydro-1,4,2-dioxazine (prepared as described in Preparation 3) was used in place of the 3-(2-chlorobenzyl)-5,6-dihydro-1,4,2-dioxazine, the title compound was obtained as a red oil in a yield of 57%.

Mass spectrum (CI, m/z): 294 ($M^+ + 3$), 214.

PREPARATION 21

2-Chloro- α -cyclopropylcarbonylbenzyl bromide

Following a procedure similar to that described in Preparation 18, except that an equivalent amount of 2-chlorobenzyl cyclopropyl ketone (prepared as described in Preparation 4) was used in place of the 3-(2-chlorobenzyl)-5,6-dihydro-1,4,2-dioxazine, the title compound was obtained as a red oil in a yield of 83%.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 0.80–1.20 (4H, multiplet); 2.04–2.16 (1H, multiplet); 6.18 (1H, singlet); 7.20–7.60 (4H, multiplet).

Mass spectrum (CI, m/z): 275 ($M^+ + 3$), 193.

PREPARATION 22

2-Chlorobenzyl 4-fluorophenyl ketone

Following a procedure similar to that described in Preparation 13, except that equivalent amounts of 2-chlorobenzyl bromide and 4-fluorobenzoyl chloride were used in place of the 2-fluorobenzyl bromide and cyclobutanecarbonyl chloride, the title compound was obtained as a colorless powder in a yield of 34%.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 4.40 (2H, singlet); 7.10–7.45 (6H, multiplet); 8.04–8.10 (2H, multiplet).

Mass spectrum (CI, m/z): 249 ($M^+ + 1$), 213.

PREPARATION 23

2-Fluorobenzyl isopropyl ketone

Following a procedure similar to that described in Preparation 4, except that equivalent amounts of 2-fluorobenzyl chloride and isobutyronitrile were used in place of the 2-chlorobenzyl bromide and cyclopropyl cyanide, the title compound was obtained as a colorless oil in a yield of 25%.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 1.15 (6H, doublet, $J=7.5$ Hz); 2.75 (1H, septet, $J=7.5$ Hz); 3.78 (2H, singlet); 6.97–7.30 (4H, multiplet).

Mass spectrum (CI, m/z): 181 ($M^+ + 1$), 109.

PREPARATION 24

2-Nitro-4,5,6,7-tetrahydrothieno[3,2-c]pyridine hydrochloride

24(a) 5-Acetyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridine

35.1 g (200 mmole) of 4,5,6,7-tetrahydrothieno[3,2-c]pyridine hydrochloride and 38.57 g (200 mmole) of 28% w/v sodium methoxide in methanol were added to 200 ml of ethanol, and the resulting mixture was stirred at room temperature for 1 hour. The inorganic salt thus precipitated was filtered off, and the filtrate was concentrated to dryness by evaporation under reduced pressure. 50 ml of acetic anhydride were added all at once, whilst stirring, to the residue, and the resulting mixture was stirred at room temperature for 1 hour. The reaction mixture was then concentrated to dryness by evaporation under reduced pressure, and the residue thus obtained was subjected to silica gel column chromatography, using a 6:4 by volume mixture of toluene and ethyl acetate as the eluent, to give 29.32 g of the title compound as a yellow oil.

24(b) 5-Acetyl-2-nitro-4,5,6,7-tetrahydrothieno[3,2-c]pyridine

20 ml of an acetic anhydride solution containing 5.43 g (30 mmole) of 5-acetyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridine [prepared as described in step (a) above]

were added dropwise at 10° to 18° C. over a period of one hour to 30 ml of an acetic acid solution containing 4.2 g (60 mmole) of 90% fuming nitric acid, and the mixture was then stirred at a temperature not greater than 18° C. for 1 hour. The reaction mixture was then poured into ice-water and extracted with methylene chloride. The organic layer was separated, washed with a saturated aqueous solution of sodium hydrogencarbonate and with water, in that order, and dried over anhydrous magnesium sulfate. The solvent was then removed by distillation under reduced pressure, and the resulting residue was crystallized from a mixture of hexane and toluene, to give 4.46 g of the title compound as yellow crystals.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 2.19 & 2.21 (together 3H, each singlet); 2.82–3.05 (2H, multiplet); 3.80 & 3.95 (together 2H, each triplet, $J=5.7$ Hz); 4.55 & 4.66 (together 2H, each singlet); 7.66 (1H, singlet).

Mass spectrum (CI, m/z): 227 ($M^+ + 1$).

24(c) 2-Nitro-4,5,6,7-tetrahydrothieno[3,2-c]pyridine hydrochloride 2.38 g (10.53 mmole) of 5-acetyl-2-nitro-4,5,6,7-tetrahydrothieno[3,2-c]pyridine [prepared as described in step (b) above] were heated under reflux for 2 hours in 60 ml of 10% w/v aqueous hydrochloric acid. The reaction mixture was then concentrated to dryness by evaporation under reduced pressure, to give 2.19 g of the title compound as brown crystals.

Nuclear Magnetic Resonance Spectrum (CD_3OD) δ ppm: 3.22 (2H, triplet, $J=6.2$ Hz); 3.60 (2H, triplet, $J=6.2$ Hz); 4.31 (2H, singlet); 7.87 (1H, singlet).

Mass spectrum (CI, m/z): 185 ($M^+ + 1$).

PREPARATION 25

4,5,6,7-Tetrahydrofuro[3,2-c]pyridine

3.7 g (46 mmole) of a 37% aqueous formaldehyde solution were added dropwise at room temperature to 5.1 g (46 mmole) of 2-furylethylamine [the compound described, for example, in Brit., J. Pharmacol., 9, 376 (1954)], and the resulting mixture was stirred for about 15 minutes, after which it was extracted with diethyl ether. The organic extract was washed with water and dried over anhydrous sodium sulfate, and then the diethyl ether was removed by distillation under reduced pressure. 5 ml of dimethylformamide were added to the residue, and the resulting solution was added dropwise to 15 ml of dimethylformamide containing 3.6 g (100 mmole) of dry hydrogen chloride at room temperature. The resulting mixture was then stirred for 3 hours. At the end of this time, the greater part of the dimethylformamide was removed by distillation under reduced pressure, and then water and a 0.1N aqueous solution of sodium hydroxide were added to the residue so as to adjust its pH to a value of about 11; the mixture was then extracted with chloroform. The organic extract was washed with water and dried over anhydrous sodium sulfate. The chloroform was then removed by evaporation under reduced pressure, and the resulting residue was purified by silica gel column chromatography, using a 50:1 by volume mixture of chloroform and methanol as the eluent, to give the title compound as a brown oil in a yield of 27%.

Nuclear Magnetic Resonance Spectrum ($CDCl_3$) δ ppm: 3.10–3.20 (4H, multiplet); 3.70–3.80 (2H, multiplet); 6.20 (1H, singlet); 7.27 (1H, singlet).

Mass spectrum (CI, m/z): 124 ($M^+ + 1$).

PREPARATION 26

2-Fluorobenzyl 2-fluorocyclopropyl ketone

A procedure similar to that described in Preparation 18 was repeated, except that an equivalent amount of 2-fluorocyclopropylcarbonyl chloride was used in place of the cyclobutylcarbonyl chloride, to give the title compound as a colorless oil in a yield of 27 %.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 1.38–1.58 (2H, multiplet); 2.34–2.56 (1H, multiplet); 3.90 (2H, singlet); 4.54–4.61 & 4.86–4.93 (together 1H, each multiplet); 7.05–7.35 (4H, multiplet).

Mass spectrum (CI, m/z): 197 (M⁺ + 1), 109.

PREPARATION 27

2-Fluoro-α-(2-fluorocyclopropylcarbonyl)benzyl bromide

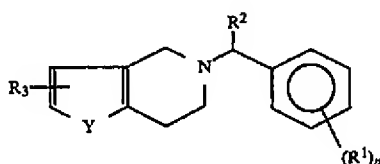
A procedure similar to that described in Preparation 18 was repeated, except that an equivalent amount of 2-fluorobenzyl 2-fluorocyclopropyl ketone was used in place of the 3-(2-chlorobenzyl)-5,6-dihydro-1,4,2-dioxazine, to give the title compound as a colorless oil in a yield of 76 %.

Nuclear Magnetic Resonance Spectrum (CDCl₃) δ ppm: 1.44–1.73 (2H, multiplet); 2.54–2.76 (1H, multiplet); 4.54–4.68 & 4.85–4.99 (together 1H, each multiplet); 5.93 (1H, singlet); 7.05–7.60 (4H, multiplet).

Mass spectrum (CI, m/z): 277 (M⁺ + 2), 275 (M⁺), 195.

We claim:

1. A compound of formula (I):



wherein

R¹ represents a hydrogen atom, an alkyl group having from 1 to 4 carbon atoms, a haloalkyl group having from 1 to 4 carbon atoms and at least one halogen atom, a hydroxy group, an alkoxy group having from 1 to 4 carbon atoms, a haloalkoxy group having from 1 to 4 carbon atoms and at least one halogen atom, an alkylthio group having from 1 to 4 carbon atoms, a haloalkylthio group having from 1 to 4 carbon atoms and at least one halogen atom, an amino group, an alkanoyl group having from 1 to 5 carbon atoms, a haloalkanoyl group having from 2 to 5 carbon atoms and at least one halogen atom, a carboxy group, an alkoxycarbonyl group having from 2 to 5 carbon atoms, a carbamoyl group, a cyano group, a nitro group, an alkanesulfonyl group having from 1 to 4 carbon atoms, a haloalkanesulfonyl group having from 1 to 4 carbon atoms and at least one halogen atom, or a sulfamoyl group;

R² represents an alkanoyl group having from 1 to 10 carbon atoms; a substituted alkanoyl group which has from 2 to 10 carbon atoms and which is substituted by at least one substituent selected from the group consisting of substituents A, defined below; an alkenoyl group having from 3 to 6 carbon atoms; a substituted alkenoyl group which has from 3 to 6 carbon atoms and which is substituted by at

least one substituent selected from the group consisting of substituents A, defined below; a cycloalkylcarbonyl group having from 4 to 8 carbon atoms; a substituted cycloalkylcarbonyl group which has from 4 to 8 carbon atoms and which is substituted by at least one substituent selected from the group consisting of substituents A, defined below; or a substituted benzoyl group having at least one substituent selected from the group consisting of substituents B, defined below;

R³ represents a hydrogen atom; a hydroxy group; an alkoxy group having from 1 to 4 carbon atoms; a substituted alkoxy group which has from 1 to 4 carbon atoms and which is substituted by at least one substituent selected from the group consisting of substituents C, defined below; an aralkyloxy group in which the aralkyl part is as defined below; an alkanoyloxy group having from 1 to 18 carbon atoms; an alkenoyloxy group having from 3 to 6 carbon atoms; a cycloalkylcarbonyloxy group having from 4 to 8 carbon atoms; an arylcarbonyloxy group in which the aryl part is as defined below; an alkoxycarbonyloxy group having from 2 to 5 carbon atoms; an aralkyloxycarbonyloxy group in which the aralkyl part is as defined below; a phthalidyl group; a (5-methyl-2-oxo-1,3-dioxol-4-yl)methoxy group; a (5-phenyl-2-oxo-1,3-dioxol-4-yl)methoxy group; a group of formula —N—R^aR^b; wherein R^a and R^b are independently selected from the group consisting of hydrogen atoms, alkyl groups having from 1 to 4 carbon atoms and substituted alkyl groups which have from 1 to 4 carbon atoms and which are substituted by at least one substituent selected from the group consisting of substituents C, defined below; an aralkylamino group in which the aralkyl part is as defined below; an alkanoylamino group having from 1 to 18 carbon atoms; an alkenoylamino group having from 3 to 6 carbon atoms; a cycloalkylcarbonylamino group having from 4 to 8 carbon atoms; an arylcarbonylamino group in which the aryl part is as defined below; an alkoxycarbonylamino group having from 2 to 5 carbon atoms; an aralkyloxycarbonylamino group in which the aralkyl part is as defined below; a phthalidylamino group; a (5-methyl-2-oxo-1,3-dioxol-4-yl)methylamino group; a (5-phenyl-2-oxo-1,3-dioxol-4-yl)methylamino group, or a nitro group;

Y is a sulfur atom; and

n is an integer from 1 to 5, and, when n is an integer from 2 to 5, the groups represented by R¹ may be the same as or different from each other;

said substituents A are selected from the group consisting of halogen atoms, hydroxy groups, alkoxy groups having from 1 to 4 carbon atoms and cyano groups;

said substituents B are selected from the group consisting of alkyl groups having from 1 to 4 carbon atoms, halogen atoms and alkoxy groups having from 1 to 4 carbon atoms;

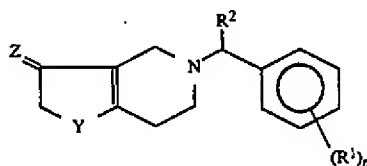
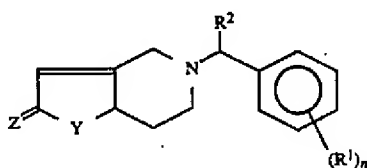
said substituents C are selected from the group consisting of alkoxy groups having from 1 to 4 carbon atoms, alkanoyloxy groups having from 1 to 6 carbon atoms and arylcarbonyloxy groups in which the aryl part is as defined below;

said aralkyl parts of said aralkyloxy, aralkyloxycarbonyloxy, aralkylamino and aralkyloxycarbonylamino groups are alkyl groups which have from 1 to 4 carbon atoms and which are substituted by at least one aryl groups as defined below;

said aryl groups and said aryl parts of said arylcarbonyloxy groups and of said arylcarbonylamino groups having from 6 to 10 carbon atoms in a carbocyclic ring which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D, defined below; and

said substituents D are selected from the group consisting of the groups and atoms defined above in relation to R¹, other than said hydrogen atom; or a tautomer thereof, or a pharmaceutically acceptable salt of said compound of formula (I) and of said tautomer.

2. The compound of claim 1, wherein said tautomer has the formula (Ia) or (Ib):



wherein R¹, R², Y and n are as defined above and Z represents group of formula =NH or an oxygen atom.

3. The compound of claim 1, wherein R¹ represents a hydrogen atom, an alkyl group having from 1 to 4 carbon atoms, a halogen atom, a fluoroalkyl group having from 1 to 4 carbon atoms and at least one fluorine atom, a hydroxy group, an alkoxy group having from 1 to 4 carbon atoms, a fluoroalkoxy group having from 1 to 4 carbon atoms and at least one fluorine atom, an alkylthio group having from 1 to 4 carbon atoms, a fluoroalkylthio group having from 1 to 4 carbon atoms and at least one fluorine atom, an amino group, an alkanoyl group having from 1 to 5 carbon atoms, a fluoroalkanoyl group having from 2 to 5 carbon atoms and at least one fluorine atom, an alkoxycarbonyl group having from 2 to 5 carbon atoms, a carbamoyl group, a cyano group, a nitro group, an alkanesulfonyl group having from 1 to 4 carbon atoms, a fluoroalkanesulfonyl group having from 1 to 4 carbon atoms and at least one fluorine atom, or a sulfamoyl group.

4. The compound of claim 1, wherein R² represents an alkanoyl group having from 2 to 6 carbon atoms, a substituted alkanoyl group which has from 2 to 6 carbon atoms and which is substituted by at least one substituent selected from the group consisting of substituents A', defined below, a cycloalkylcarbonyl group having from 4 to 7 carbon atoms, a substituted cycloalkylcarbonyl group which has from 4 to 7 carbon atoms and which is substituted by at least one substituent selected from the group consisting of substituents A',

defined below of a substituted benzoyl group having at least one fluorine substituent; and

said substituents A' are selected from the group consisting of fluorine atoms, chlorine atoms, hydroxy groups, methoxy groups, ethoxy groups and cyano groups.

5. The compound of claim 1, wherein:

R³ represents a hydrogen atom, a hydroxy group, an alkoxy group having from 1 to 4 carbon atoms, an alkoxymethoxy group in which the alkoxy part has from 1 to 4 carbon atoms, an alkanoyloxymethoxy group in which the alkanoyl part has from 1 to 5 carbon atoms, a benzyloxy group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D', defined below, an alkanoyloxy group having from 1 to 18 carbon atoms, an alkenoyloxy group having 3 or 4 carbon atoms, a cycloalkylcarbonyloxy group having from 4 to 7 carbon atoms, a benzoyloxy group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D', defined below, an alkoxycarbonyloxy group having from 2 to 5 carbon atoms, a benzyloxy carbonyloxy group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D', defined below, a phthalidylloxy group, a (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy group, a (5-phenyl-2-oxo-1,3-dioxolen-4-yl)methoxy group, a group of formula —NR^aR^b wherein R^a and R^b are independently selected from the group consisting of hydrogen atoms, methyl and ethyl groups or R^a represents a hydrogen atom and R^b represents an alkanoyloxymethyl group in which the alkanoyl part has from 1 to 5 carbon atoms,

a benzylamino group, an alkanoylamino group having from 1 to 18 carbon atoms, an alkenoylamino group having 3 or 4 carbon atoms, a cycloalkylcarbonylamino group having 6 or 7 carbon atoms, a benzoylamino group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D', defined below, an alkoxycarbonylamino group having from 2 to 5 carbon atoms or a benzyloxycarbonylamino group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D', defined below; and

said substituents D' are selected from the group consisting of fluorine atoms, chlorine atoms, methyl groups and methoxy groups.

6. The compound of claim 1, wherein:

R¹ represents a hydrogen atom, an alkyl group having from 1 to 4 carbon atoms, a halogen atom, a fluoroalkyl group having from 1 to 4 carbon atoms and at least one fluorine atom, a hydroxy group, an alkoxy group having from 1 to 4 carbon atoms, a fluoroalkoxy group having from 1 to 4 carbon atoms and at least one fluorine atom, an alkylthio group having from 1 to 4 carbon atoms, a fluoroalkylthio group having from 1 to 4 carbon atoms and at least one fluorine atom, an amino group, an alkanoyl group having from 1 to 5 carbon atoms, a fluoroalkanoyl group having from 2 to 5 carbon atoms and at least one fluorine atom, an alkoxycarbonyl group having from 2 to 5 carbon atoms, a carbamoyl group, a cyano group, a nitro group, an

alkanesulfonyl group having from 1 to 4 carbon atoms, a fluoroalkanesulfonyl group having from 1 to 4 carbon atoms and at least one fluorine atom, or a sulfamoyl group;

R^2 represents an alkanoyl group having from 2 to 6 carbon atoms, a substituted alkanoyl group which has from 2 to 6 carbon atoms and which is substituted by at least one substituent selected from the group consisting of substituents A' , defined below, a cycloalkylcarbonyl group having from 4 to 7 carbon atoms, a substituted cycloalkylcarbonyl group which has from 4 to 7 carbon atoms and which is substituted by at least one substituent selected from the group consisting of substituents A' , defined below or a substituted benzoyl group having at least one fluorine substituent;

R^3 represents a hydrogen atom, a hydroxy group, an alkoxy group having from 1 to 4 carbon atoms, an alkoxymethoxy group in which the alkoxy part has from 1 to 4 carbon atoms, an alkanoyloxymethoxy group in which the alkanoyl part has from 1 to 5 carbon atoms, a benzyloxy group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D' , defined below, an alkanoyloxy group having from 1 to 18 carbon atoms, an alkenoyloxy group having 3 or 4 carbon atoms, a cycloalkylcarbonyloxy group having from 4 to 7 carbon atoms, a benzoyloxy group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D' , defined below, an alkoxycarbonyloxy group having from 2 to 5 carbon atoms, a benzyloxycarbonyloxy group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D' , defined below, a phthalidylloxy group, a (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy group, a (5-phenyl-2-oxo-1,3-dioxolen-4-yl)methoxy group, a group of formula $-NR^aR^b$ wherein R^a and R^b are independently selected from the group consisting of hydrogen atoms, methyl groups and ethyl groups or R^a represents a hydrogen atom and R^b represents an alkanoyloxymethyl group in which the alkanoyl part has from 1 to 5 carbon atoms,

a benzylamino group, an alkanoylamino group having from 1 to 18 carbon atoms, an alkenoylamino group having 3 or 4 carbon atoms, a cycloalkylcarbonylamino group having 6 or 7 carbon atoms, a benzoylamino group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D' , defined below, an alkoxycarbonylamino group having from 2 to 5 carbon atoms or a benzyloxycarbonylamino group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D' , defined below;

said substituents A' are selected from the group consisting of fluorine atoms, chlorine atoms, hydroxy groups, methoxy groups, ethoxy groups and cyano groups; and

said substituents D' are selected from the group consisting of fluorine atoms, chlorine atoms, methyl groups and methoxy groups.

7. The compound of claim 6, wherein n is from 1 to 3.

8. The compound of claim 6, wherein n is 1.

9. The compound of claim 1, wherein R^1 represents a hydrogen atom, a methyl group, an ethyl group, a halo-

gen atom, a methyl group substituted by at least one fluorine atom, a hydroxy group, a methoxy group, an ethoxy group, a methoxy group substituted by at least one fluorine atom, a methylthio group, a methylthio group substituted by at least one fluorine atom, a formyl group, an acetyl group, an acetyl group substituted by at least one fluorine atom, an alkoxycarbonyl group having from 2 to 4 carbon atoms, a carbamoyl group, a cyano group, a nitro group, a methanesulfonyl group, an ethanesulfonyl group, a methanesulfonyl group substituted by at least one fluorine atom, or a sulfamoyl group.

10. The compound of claim 1, wherein R^2 represents an alkanoyl group having from 2 to 6 carbon atoms, a substituted alkanoyl group which has from 2 to 6 carbon atoms and which is substituted by at least one fluorine atom, a cycloalkylcarbonyl group having from 4 to 7 carbon atoms, or a substituted cycloalkylcarbonyl group which is substituted by at least one fluorine atom.

11. The compound of claim 1, wherein R^3 represents a hydrogen atom, a hydroxy group, a methoxy group, an ethoxy group, a *t*-butoxy group, a methoxymethoxy group, an alkanoyloxymethoxy group in which the alkanoyl part has from 1 to 5 carbon atoms, a benzyloxy group, an alkanoyloxy group having from 1 to 12 carbon atoms, an alkenoyloxy group having 3 or 4 carbon atoms, a cycloalkylcarbonyloxy group having from 4 to 7 carbon atoms, a benzoyloxy group, an alkoxycarbonyloxy group having from 2 to 5 carbon atoms, a benzyloxycarbonyloxy group, a phthalidylloxy group, a (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy group, a (5-phenyl-2-oxo-1,3-dioxolen-4-yl)methoxy group, an amino group or a *t*-butoxycarbonylamino group.

12. The compound of claim 1, wherein:

R^1 represents a hydrogen atom, a methyl group, an ethyl group, a halogen atom, a methyl group substituted by at least one fluorine atom, a hydroxy group, a methoxy group, an ethoxy group, a methoxy group substituted by at least one fluorine atom, a methylthio group, a methylthio group substituted by at least one fluorine atom, a formyl group, an acetyl group, an acetyl group substituted by at least one fluorine atom, an alkoxycarbonyl group having from 2 to 4 carbon atoms, a carbamoyl group, a cyano group, a nitro group, a methanesulfonyl group, an ethanesulfonyl group, a methanesulfonyl group substituted by at least one fluorine atom, or a sulfamoyl group;

R^2 represents an alkanoyl group having from 2 to 6 carbon atoms, a substituted alkanoyl group which has from 2 to 6 carbon atoms and which is substituted by at least one fluorine atom, a cycloalkylcarbonyl group having from 4 to 7 carbon atoms, or a substituted cycloalkylcarbonyl group which is substituted by at least one fluorine atom; and

R^3 represents a hydrogen atom, a hydroxy group, a methoxy group, an ethoxy group, a *t*-butoxy group, a methoxymethoxy group, an alkanoyloxymethoxy group in which the alkanoyl part has from 1 to 5 carbon atoms, a benzyloxy group, an alkanoyloxy group having from 1 to 12 carbon atoms, an alkenoyloxy group having 3 or 4 carbon atoms, a cycloalkylcarbonyloxy group having from 4 to 7 carbon atoms, a benzoyloxy group, an alkoxycarbonyloxy group having from 2 to 5 carbon atoms, a benzyloxycarbonyloxy group, a phthalidylloxy group, a (5-methyl-2-oxo-1,3-dioxolen-4-yl)me-

- thoxy group, a (5-phenyl-2-oxo-1,3-dioxolen-4-yl)methoxy group, an amino group or a t-butoxycarbonylamino group.
13. The compound of claim 12, wherein n is from 1 to 3.
14. The compound of claim 12, wherein n is 1.
15. The compound of claim 1, wherein R¹ represents a halogen atom, a trifluoromethyl group, a hydroxy group, a difluoromethoxy group, a trifluoromethoxy group, a difluoromethylthio group, a trifluoromethylthio group, a formyl group, an acetyl group, a trifluoroacetyl group, a cyano group or a nitro group.
16. The compound of claim 1, wherein R³ represents a hydrogen atom, a hydroxy group, a pivaloyloxymethoxy group, an alkanoyloxy group having from 2 to 10 carbon atoms, an alkoxycarbonyloxy group having from 2 to 5 carbon atoms or a (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy group.
17. The compound of claim 1, wherein:
 R¹ represents a halogen atom, a trifluoromethyl group, a hydroxy group, a difluoromethoxy group, a trifluoromethoxy group, a difluoromethylthio group, a trifluoromethylthio group, a formyl group, an acetyl group, a trifluoroacetyl group, a cyano group or a nitro group;
 R² represents an alkanoyl group having from 2 to 6 carbon atoms, a substituted alkanoyl group which has from 2 to 6 carbon atoms and which is substituted by at least one fluorine atom, a cycloalkylcarbonyl group having from 4 to 7 carbon atoms, or a substituted cycloalkylcarbonyl group which is substituted by at least one fluorine atom; and
 R³ represents a hydrogen atom, a hydroxy group, a pivaloyloxymethoxy group, an alkanoyloxy group having from 2 to 10 carbon atoms, an alkoxycarbonyloxy group having from 2 to 5 carbon atoms or a (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy group.
18. The compound of claim 17, wherein n is from 1 to 3.
19. The compound of claim 17, wherein n is 1.
20. The compound of claim 1, wherein R² represents an acetyl group, a propionyl group, a substituted acetyl or propionyl group which is substituted by at least one fluorine atom, a cyclopropylcarbonyl group, cyclobutylcarbonyl group, or a substituted cyclopropylcarbonyl or cyclobutylcarbonyl group which is substituted by at least one fluorine atom.
21. The compound of claim 1, wherein R³ represents a hydrogen atom, a hydroxy group, a pivaloyloxymethoxy group, an alkanoyloxy group having from 2 to 6 carbon atoms or an alkoxycarbonyloxy group having from 2 to 5 carbon atoms.
22. The compound of claim 1, wherein:
 R¹ represents a fluorine or chlorine atom;
 R² represents an acetyl group, a propionyl group, a substituted acetyl or propionyl group which is substituted by at least one fluorine atom, a cyclopropylcarbonyl group, cyclobutylcarbonyl group, or a substituted cyclopropylcarbonyl or cyclobutylcarbonyl group which is substituted by at least one fluorine atom; and
 R³ represents a hydrogen atom, a hydroxy group, a pivaloyloxymethoxy group, an alkanoyloxy group having from 2 to 6 carbon atoms or an alkoxycarbonyloxy group having from 2 to 5 carbon atoms.
23. The compound of claim 22, wherein n is from 1 to 3.

24. The compound of claim 22, wherein n is 1.
25. The compound of claim 1, selected from the group consisting of 5-(2-fluoro- α -propionylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and pharmaceutically acceptable salts thereof.
26. The compound of claim 1, selected from the group consisting of 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and pharmaceutically acceptable salts thereof.
27. The compound of claim 1, selected from the group consisting of 5-(2-chloro- α -cyclopropylcarbonylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and pharmaceutically acceptable salts thereof.
28. The compound of claim 1, selected from the group consisting of 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and pharmaceutically acceptable salts thereof.
29. The compound of claim 1, selected from the group consisting of 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-propionyloxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and pharmaceutically acceptable salts thereof.
30. The compound of claim 1, selected from the group consisting of 2-butyryloxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and pharmaceutically acceptable salts thereof.
31. The compound of claim 1, selected from the group consisting of 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-pivaloyloxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and pharmaceutically acceptable salts thereof.
32. The compound of claim 1, selected from the group consisting of 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-valeryloxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and pharmaceutically acceptable salts thereof.
33. The compound of claim 1, selected from the group consisting of 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-hexanoyloxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and pharmaceutically acceptable salts thereof.
34. The compound of claim 1, selected from the group consisting of 2-t-butoxycarbonyloxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and pharmaceutically acceptable salts thereof.
35. The compound of claim 1, selected from the group consisting of 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-pivaloyloxymethoxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and pharmaceutically acceptable salts thereof.
36. The compound of claim 1, selected from the group consisting of 5-(2-chloro- α -cyclopropylcarbonylbenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine and its tautomer and pharmaceutically acceptable salts thereof.
37. The compound of claim 1, selected from the group consisting of 5-(2-fluoro- α -propionylbenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine and its tautomer and pharmaceutically acceptable salts thereof.
38. The compound of claim 1, selected from the group consisting of 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine and its tautomer and pharmaceutically acceptable salts thereof.
39. The compound of claim 1, selected from the group consisting of 2-acetoxy-5-(2-chloro- α -cyclo-

propylcarbonylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and pharmaceutically acceptable salts thereof.

40. The compound of claim 1, selected from the group consisting of 5-[α -(2-fluorocyclopropylcarbonyl-2-fluorobenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine and its tautomer and pharmaceutically acceptable salts thereof.

41. The compound of claim 1, selected from the group consisting of 2-acetoxy-5-[α -(2-fluorocyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and pharmaceutically acceptable salts thereof.

42. A pharmaceutical composition for the treatment and prophylaxis of thrombosis or embolisms, comprising an effective amount of a blood platelet aggregation inhibitor in admixture with a pharmaceutically acceptable carrier or diluent, wherein said inhibitor is at least one compound of formula (I), or a tautomer or pharmaceutically acceptable salt thereof, as claimed in claim 1.

43. The composition of claim 42, wherein:

R¹ represents a hydrogen atom, an alkyl group having from 1 to 4 carbon atoms, a halogen atom, a fluoroalkyl group having from 1 to 4 carbon atoms and at least one fluorine atom, a hydroxy group, an alkoxy group having from 1 to 4 carbon atoms, a fluoroalkoxy group having from 1 to 4 carbon atoms and at least one fluorine atom, an alkylthio group having from 1 to 4 carbon atoms, a fluoroalkylthio group having from 1 to 4 carbon atoms and at least one fluorine atom, an amino group, an alkanoyl group having from 1 to 5 carbon atoms, a fluoroalkanoyl group having from 2 to 5 carbon atoms and at least one fluorine atom, an alkoxy carbonyl group having from 2 to 5 carbon atoms, a carbamoyl group, a cyano group, a nitro group, an alkanesulfonyl group having from 1 to 4 carbon atoms, a fluoroalkanesulfonyl group having from 1 to 4 carbon atoms and at least one fluorine atom, or a sulfamoyl group;

R² represents an alkanoyl group having from 2 to 6 carbon atoms, a substituted alkanoyl group which has from 2 to 6 carbon atoms and which is substituted by at least one substituent selected from the group consisting of substituents A', defined below, a cycloalkylcarbonyl group having from 4 to 7 carbon atoms, a substituted cycloalkylcarbonyl group which has from 4 to 7 carbon atoms and which is substituted by at least one substituent selected from the group consisting of substituents A', defined below, or a substituted benzoyl group having at least one fluorine substituent, or a;

R³ represents a hydrogen atom, a hydroxy group, an alkoxy group having from 1 to 4 carbon atoms, an alkoxy methoxy group in which the alkoxy part has from 1 to 4 carbon atoms, an alkanoyloxymethoxy group in which the alkanoyl part has from 1 to 5 carbon atoms, a benzyloxy group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D', defined below, an alkanoyloxy group having from 1 to 18 carbon atoms, an alkenoyloxy group having 3 or 4 carbon atoms, a cycloalkylcarbonyloxy group having from 4 to 7 carbon atoms, a benzoyloxy group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D', defined below, an alkoxy carbonyloxy group having from 2

to 5 carbon atoms, a benzyloxy carbonyloxy group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D', defined below, a phthalidyloxy group, a (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy group, a (5-phenyl-2-oxo-1,3-dioxolen-4-yl)methoxy group, a group of formula —NR^aR^b wherein R^a and R^b are independently selected from the group consisting of hydrogen atoms, methyl groups and ethyl groups or R^a represents a hydrogen atom and R^b represents an alkanoyloxymethyl group in which the alkanoyl part has from 1 to 5 carbon atoms,

a benzylamino group, an alkanoylamino group having from 1 to 18 carbon atoms, an alkenoylamino group having 3 or 4 carbon atoms, a cycloalkylcarbonylamino group having 6 or 7 carbon atoms, a benzoylamino group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D', defined below, an alkoxy carbonylamino group having from 2 to 5 carbon atoms or a benzyloxy carbonylamino group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D', defined below;

said substituents A' are selected from the group consisting of fluorine atoms, chlorine atoms, hydroxy groups, methoxy groups, ethoxy groups and cyano groups; and

said substituents D' are selected from the group consisting of fluorine atoms, chlorine atoms, methyl groups and methoxy groups.

44. The composition of claim 42, wherein:

R¹ represents a hydrogen atom, a methyl group, an ethyl group, a halogen atom, a methyl group substituted by at least one fluorine atom, a hydroxy group, a methoxy group, an ethoxy group, a methoxy group substituted by at least one fluorine atom, a methylthio group, a methylthio group substituted by at least one fluorine atom, a formyl group, an acetyl group, an acetyl group substituted by at least one fluorine atom, an alkoxy carbonyl group having from 2 to 4 carbon atoms, a carbamoyl group, a cyano group, a nitro group, a methanesulfonyl group, an ethanesulfonyl group, a methanesulfonyl group substituted by at least one fluorine atom, or a sulfamoyl group;

R² represents an alkanoyl group having from 2 to 6 carbon atoms, a substituted alkanoyl group which has from 2 to 6 carbon atoms and which is substituted by at least one fluorine atom, a cycloalkylcarbonyl group having from 4 to 7 carbon atoms, or a substituted cycloalkylcarbonyl group which is substituted by at least one fluorine atom; and

R³ represents a hydrogen atom, a hydroxy group, a methoxy group, an ethoxy group, a t-butoxy group, a methoxymethoxy group, an alkanoyloxymethoxy group in which the alkanoyl part has from 1 to 5 carbon atoms, a benzyloxy group, an alkanoyloxy group having from 1 to 12 carbon atoms, an alkenoyloxy group having 3 or 4 carbon atoms, a cycloalkylcarbonyloxy group having from 4 to 7 carbon atoms, a benzoyloxy group, an alkoxy carbonyloxy group having from 2 to 5 carbon atoms, a benzyloxy carbonyloxy group, a phthalidyloxy group, a (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy group, a (5-phenyl-2-oxo-1,3-dioxolen-4-

yl)methoxy group, an amino group or a t-butoxycarbonylamino group,
Y represents an oxygen or sulfur atom.

45. The composition of claim 42, wherein:

R¹ represents a halogen atom, a trifluoromethyl group, a hydroxy group, a difluoromethoxy group, a trifluoromethoxy group, a difluoromethylthio group, a trifluoromethylthio group, a formyl group, an acetyl group, a trifluoroacetyl group, a cyano group or a nitro group;

R² represents an alkanoyl group having from 2 to 6 carbon atoms, a substituted alkanoyl group which has from 2 to 6 carbon atoms and which is substituted by at least one fluorine atom, a cycloalkylcarbonyl group having from 4 to 7 carbon atoms, or a substituted cycloalkylcarbonyl group which is substituted by at least one fluorine atom; and

R³ represents a hydrogen atom, a hydroxy group, a pivaloyloxymethoxy group, an alkanoyloxy group having from 2 to 10 carbon atoms, an alkoxy carbonyloxy group having from 2 to 5 carbon atoms or a (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy group;

46. The composition of claim 42, wherein:

R¹ represents a fluorine or chlorine atom;

R² represents an acetyl group, a propionyl group, a substituted acetyl or propionyl group which is substituted by at least one fluorine atom, a cyclopropylcarbonyl group, cyclobutylcarbonyl group, or a substituted cyclopropylcarbonyl or cyclobutylcarbonyl group which is substituted by at least one fluorine atom; and

R³ represents a hydrogen atom, a hydroxy group, a pivaloyloxymethoxy group, an alkanoyloxy group having from 2 to 6 carbon atoms or an alkoxy carbonyloxy group having from 2 to 5 carbon atoms.

47. The composition of claim 42, wherein said blood platelet aggregation inhibitor is selected from the group consisting of:

- 5-(2-fluoro- α -propionylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
- 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
- 5-(2-chloro- α -cyclopropylcarbonylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
- 2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
- 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-propionyloxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
- 2-butyryloxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
- 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-pivaloyloxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
- 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-valeryloxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
- 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-hexanoyloxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
- 2-t-butoxycarbonyloxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
- 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-pivaloyloxymethoxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
- 5-(2-chloro- α -cyclopropylcarbonylbenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine and its tautomer;

5-(2-fluoro- α -propionylbenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine and its tautomer;
5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine and its tautomer;

2-acetoxy-5-(2-chloro- α -cyclopropylcarbonylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;

5[α -(2-fluorocyclopropylcarbonyl-2-fluorobenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine and its tautomer;

2-acetoxy-5-[α -(2-fluorocyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;

and pharmaceutically acceptable salts thereof.

48. A method for the treatment or prophylaxis of thrombosis or embolisms, comprising administering to a mammal an effective amount of a blood platelet aggregation inhibitor, wherein said inhibitor is at least one compound of formula (I), or a tautomer or pharmaceutically acceptable salt thereof, as claimed in claim 1.

49. The method of claim 48, wherein:

R¹ represents a hydrogen atom, an alkyl group having from 1 to 4 carbon atoms, a halogen atom, a fluoroalkyl group having from 1 to 4 carbon atoms and at least one fluorine atom, a hydroxy group, an alkoxy group having from 1 to 4 carbon atoms, a fluoroalkoxy group having from 1 to 4 carbon atoms and at least one fluorine atom, an alkylthio group having from 1 to 4 carbon atoms, a fluoroalkylthio group having from 1 to 4 carbon atoms and at least one fluorine atom, an amino group, an alkanoyl group having from 1 to 5 carbon atoms, a fluoroalkanoyl group having from 2 to 5 carbon atoms and at least one fluorine atom, an alkoxy carbonyl group having from 2 to 5 carbon atoms, a carbamoyl group, a cyano group, a nitro group, an alkanesulfonyl group having from 1 to 4 carbon atoms, a fluoroalkanesulfonyl group having from 1 to 4 carbon atoms and at least one fluorine atom, or a sulfamoyl group;

R² represents an alkanoyl group having from 2 to 6 carbon atoms, a substituted alkanoyl group which has from 2 to 6 carbon atoms and which is substituted by at least one substituent selected from the group consisting of substituents A', defined below, a cycloalkylcarbonyl group having from 4 to 7 carbon atoms, a substituted cycloalkylcarbonyl group which has from 4 to 7 carbon atoms and which is substituted by at least one substituent selected from the group consisting of substituents A', defined below, of a substituted benzoyl group having at least one fluorine substituent;

R³ represents a hydrogen atom, a hydroxy group, an alkoxy group having from 1 to 4 carbon atoms, an alkoxy methoxy group in which the alkoxy part has from 1 to 4 carbon atoms, an alkanoyloxymethoxy group in which the alkanoyl part has from 1 to 5 carbon atoms, a benzyloxy group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D', defined below, an alkanoyloxy group having from 1 to 18 carbon atoms, an alkenoyloxy group having 3 or 4 carbon atoms, a cycloalkylcarbonyloxy group having from 4 to 7 carbon atoms, a benzyloxy group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D', defined below, an alkoxy carbonyloxy group having from 2

to 5 carbon atoms, a benzyloxycarbonyloxy group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D', defined below, a phthalidyloxy group, a (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy group, a (5-phenyl-2-oxo-1,3-dioxolen-4-yl)methoxy group, a group of formula $-NR^aR^b$ wherein R^a and R^b are independently selected from the group consisting of hydrogen atoms, methyl groups and ethyl groups or R^a represents a hydrogen atom and R^b represents an alkanoyloxymethyl group in which the alkanoyl part has from 1 to 5 carbon atoms, a benzylamino group, an alkanoylamino group having from 1 to 18 carbon atoms, an alkenoylamino group having 3 or 4 carbon atoms, a cycloalkylcarbonylamino group having 6 or 7 carbon atoms, a benzoylamino group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D', defined below, an alkoxycarbonylamino group having from 2 to 5 carbon atoms or a benzyloxycarbonylamino group which is unsubstituted or is substituted by at least one substituent selected from the group consisting of substituents D', defined below;

said substituents A' are selected from the group consisting of fluorine atoms, chlorine atoms, hydroxy groups, methoxy groups, ethoxy groups and cyano groups; and

said substituents D' are selected from the group consisting of fluorine atoms, chlorine atoms, methyl groups and methoxy groups.

50. The method of claim 48, wherein:

R¹ represents a hydrogen atom, a methyl group, an ethyl group, a halogen atom, a methyl group substituted by at least one fluorine atom, a hydroxy group, a methoxy group, an ethoxy group, a methoxy group substituted by at least one fluorine atom, a methylthio group, a methylthio group substituted by at least one fluorine atom, a formyl group, an acetyl group, an acetyl group substituted by at least one fluorine atom, an alkoxycarbonyl group having from 2 to 4 carbon atoms, a carbamoyl group, a cyano group, a nitro group, a methanesulfonyl group, an ethanesulfonyl group, a methanesulfonyl group substituted by at least one fluorine atom, or a sulfamoyl group;

R² represents an alkanoyl group having from 2 to 6 carbon atoms, a substituted alkanoyl group which has from 2 to 6 carbon atoms and which is substituted by at least one fluorine atom, a cycloalkylcarbonyl group having from 4 to 7 carbon atoms, or a substituted cycloalkylcarbonyl group which is substituted by at least one fluorine atom; and

R³ represents a hydrogen atom, a hydroxy group, a methoxy group, an ethoxy group, a t-butoxy group, a methoxymethoxy group, an alkanoyloxymethoxy group in which the alkanoyl part has from 1 to 5 carbon atoms, a benzyloxy group, an alkanoyloxy group having from 1 to 12 carbon atoms, an alkenoyloxy group having 3 or 4 carbon atoms, a cycloalkylcarbonyloxy group having from 4 to 7 carbon atoms, a benzoyloxy group, an alkoxycarbonyloxy group having from 2 to 5 carbon atoms, a benzyloxycarbonyloxy group, a phthalidyloxy group, a (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy group, a (5-phenyl-2-oxo-1,3-dioxolen-4-

yl)methoxy group, an amino group or a t-butoxycarbonylamino group.

51. The method of claim 48, wherein:

R¹ represents a halogen atom, a trifluoromethyl group, a hydroxy group, a difluoromethoxy group, a trifluoromethoxy group, a difluoromethylthio group, a trifluoromethylthio group, a formyl group, an acetyl group, a trifluoroacetyl group, a cyano group or a nitro group;

R² represents an alkanoyl group having from 2 to 6 carbon atoms, a substituted alkanoyl group which has from 2 to 6 carbon atoms and which is substituted by at least one fluorine atom, a cycloalkylcarbonyl group having from 4 to 7 carbon atoms, or a substituted cycloalkylcarbonyl group which is substituted by at least one fluorine atom; and

R³ represents a hydrogen atom, a hydroxy group, a pivaloyloxymethoxy group, an alkanoyloxy group having from 2 to 10 carbon atoms, an alkoxycarbonyloxy group having from 2 to 5 carbon atoms or a (5-methyl-2-oxo-1,3-dioxolen-4-yl)methoxy group.

52. The method of claim 48, wherein:

R¹ represents a fluorine or chlorine atom;

R² represents an acetyl group, a propionyl group, a substituted acetyl or propionyl group which is substituted by at least one fluorine atom, a cyclopropylcarbonyl group, cyclobutylcarbonyl group, or a substituted cyclopropylcarbonyl or cyclobutylcarbonyl group which is substituted by at least one fluorine atom;

R³ represents a hydrogen atom, a hydroxy group, a pivaloyloxymethoxy group, an alkanoyloxy group having from 2 to 6 carbon atoms or an alkoxycarbonyloxy group having from 2 to 5 carbon atoms; and

Y represents a sulfur atom.

53. The method of claim 48, wherein said blood platelet aggregation inhibitor is selected from the group consisting of:

5-(2-fluoro- α -propionylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;

5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;

5-(2-chloro- α -cyclopropylcarbonylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;

2-acetoxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;

5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-propionyloxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;

2-butyryloxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;

5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-pivaloyloxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;

5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-valeryloxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;

5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-hexanoyloxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;

2-t-butoxycarbonyloxy-5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;

5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-pivaloyloxymethoxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;

5-(2-chloro- α -cyclopropylcarbonylbenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine and its tautomer;
 5-(2-fluoro- α -propionylbenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine and its tautomer;
 5-(α -cyclopropylcarbonyl-2-fluorobenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine and its tautomer;
 2-acetoxy-5-(2-chloro- α -cyclopropylcarbonylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
 5-[α -(2-fluorocyclopropylcarbonyl-2-fluorobenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine and its tautomer;
 2-acetoxy-5-[α -(2-fluorocyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine;
 and pharmaceutically acceptable salts thereof.

54. The compound of claim 1, wherein R¹ represents a fluorine atom.
 55. The compound of claim 1, wherein R¹ represents a chlorine atom.
 56. The compound of claim 1, wherein R¹ represents a fluorine atom;
 R² represents an acetyl group, a propionyl group, a substituted acetyl or propionyl group which is substituted by at least one fluorine atom, a cyclopropylcarbonyl group, cyclobutylcarbonyl group, or a substituted cyclopropylcarbonyl or cyclobutylcarbonyl group which is substituted by at least one fluorine atom;
 R³ represents a hydrogen atom, a hydroxy group, a privaloyloxymethoxy group, an alkanoyloxy group having from 2 to 6 carbon atoms or an alkoxycarbonyloxy group having from 2 to 5 carbon atoms; and
 Y represents a sulfur atom.

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UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,288,726
DATED : February 22, 1994
INVENTOR(S) : Koike et al

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 68, line 1: delete "of" and insert --, or--.

Column 69, line 15: after "below" insert -- , --.

Column 75, lines 2 and 3: after "group" delete ", Y represents an oxygen or sulfur atom".

Column 75, line 23: delete ";" and insert -- . --.

Column 76, line 51, after "below," delete "of" and insert --or--.

Column 78, line 32, after ";" insert --and--.

Column 78, lines 36-38, delete "; and Y represents a sulfur atom".

Signed and Sealed this
Twenty-first Day of April, 1998



Attest:

BRUCE LEHMAN

Attesting Officer

Commissioner of Patents and Trademarks

Exhibit 5

Platelet Reactivity in Patients and Recurrent Events Post-Stenting: Results of the PREPARE POST-STENTING Study
Paul A. Gurbel, Kevin P. Bliden, Kirk Guyer, Peter W. Cho, Kazi A. Zaman, Rolf P. Kreutz, Ashwani K. Bassi, and Udaya S. Tantry
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The online version of this article, along with updated information and services, is located on the World Wide Web at:
<http://content.onlinejacc.org/cgi/content/full/46/10/1820>

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JOURNAL of the AMERICAN COLLEGE of CARDIOLOGY



Platelets and Stent Thrombosis

Platelet Reactivity in Patients and Recurrent Events Post-Stenting

Results of the PREPARE POST-STENTING Study

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Baltimore, Maryland; and South Bend, Indiana

OBJECTIVES	We investigated the relation of high ex vivo platelet reactivity, rapid fibrin generation, and high thrombin-induced clot strength to postdischarge ischemic events in patients undergoing percutaneous coronary intervention (PCI).
BACKGROUND	High platelet reactivity and rapid fibrin generation may affect the incidence of ischemic events after PCI. However, limited data is available to link these ex vivo markers to the occurrence of events.
METHODS	We measured platelet reactivity to adenosine diphosphate (ADP) by light transmittance aggregometry (LTA) in patients undergoing PCI (n = 192). Clot strength, a measure of thrombin-induced fibrin and platelet interactions, and the time to initial fibrin generation, a marker of thrombin activity, were measured by thrombelastography. The relation of these measurements to ischemic event occurrence was prospectively examined over six months.
RESULTS	A total of 100% and 84% of patients were on aspirin and clopidogrel therapy, respectively, at the time of the initial event. Posttreatment ADP-induced aggregation by LTA ($63 \pm 12\%$ vs. $56 \pm 15\%$, $p = 0.02$) and clot strength (MA) were higher (74 ± 5 mm vs. 65 ± 4 mm, $p < 0.001$) and time to initial fibrin generation was shorter (4.3 ± 1.3 min vs. 5.9 ± 1.5 min, $p < 0.001$) in patients with events (n = 38). The event rates in the highest quartiles of LTA and MA were 32% and 58%, respectively.
CONCLUSIONS	High platelet reactivity and clot strength, and rapid fibrin formation are novel risk factors for ischemic events after PCI. Clot strength is more predictive than ADP-induced platelet aggregation and may explain the occurrence of events despite treatment with cyclooxygenase-1 and P2Y ₁₂ inhibitors. (J Am Coll Cardiol 2005;46:1820–6) © 2005 by the American College of Cardiology Foundation

Platelet aggregation and activation mediated by various agonists play fundamental roles in the development of ischemia in patients with coronary artery diseases (1). Activated platelets mediate vessel wall inflammation, and generation of thrombin and platelet-platelet aggregates mechanically obstruct the vessel lumen (2). These fundamental properties served as the rationale for the evaluation of dual antiplatelet therapy in two major clinical trials (3,4). The Clopidogrel for the Reduction of Events During Observation (CREDO) and Percutaneous Coronary Intervention-Clopidogrel in Unstable angina to prevent Recurrent ischemic Events (PCI-CURE) studies clearly demonstrated the beneficial effects of dual antiplatelet therapy after percutaneous coronary intervention in patients with unstable coronary artery syndromes and provided strong support for the clinical significance of platelet inhibition. However, recurrent ischemic events occurred in 8.5% to 8.8% of patients despite dual antiplatelet therapy (3,4).

Because ischemic events are strongly influenced by platelet-mediated events, it is logical to hypothesize that patients suffering these events will have greater ex vivo platelet reactivity than those without events despite the use of antiplatelet drugs (5). A major reason for the lack of data correlating individual platelet function to the occurrence of ischemic events is the tedious nature, labor, and expense of serial testing with conventional laboratory assays (6). Adenosine diphosphate (ADP), thromboxane A₂, and thrombin are important in vivo platelet agonists (1). Among patients undergoing percutaneous coronary intervention (PCI), patients with high platelet reactivity to low concentration ADP and lowest inhibition of ADP-induced platelet aggregation had the greatest incidence of ischemic events after the procedure (7,8). In addition, the physical properties of the clot and the kinetics of thrombin-dependent fibrin generation may affect the occurrence of ischemic events. We hypothesized that high ex vivo platelet reactivity, rapid fibrin generation, and high thrombin-induced clot strength (MA) as measured by the Thrombelastograph (TEG) Hemostasis Analyzer (Haemoscope Corporation, Niles, Illinois) were risk factors for postdischarge ischemic events in patients undergoing PCI (9–12).

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Abbreviations and Acronyms

ADP	=	adenosine diphosphate
GP	=	glycoprotein
LTA	=	light transmittance aggregometry
MA	=	clot strength
PCI	=	percutaneous coronary intervention
PPP	=	platelet-poor plasma
PRP	=	platelet-rich plasma
R	=	reaction time
ROC	=	receiver operator curve
TEG	=	thrombelastograph
TVR	=	target vessel revascularization

METHODS

Patients. The Investigational Review Board at Sinai Hospital of Baltimore approved this study. Consecutive patients undergoing nonemergent coronary stenting provided informed consent before the procedure. Study inclusion required that patients had to undergo percutaneous revascularization and be discharged from the hospital. Patients who provided informed consent but received coronary bypass surgery for revascularization were excluded. All patients were >18 years old. Other exclusion criteria were a history of bleeding diathesis, acute myocardial infarction within 48 h, elevated cardiac markers (above upper limits normal for the respective assay), cerebrovascular event within 3 months, chronic vessel occlusion or angiographically visible thrombus, illicit drug or alcohol abuse, prothrombin time greater than $1.5\times$ control, platelet count $<100,000/\text{mm}^3$, hematocrit $<30\%$, creatinine >4.0 mg/dl, and glycoprotein (GP) IIb/IIIa inhibitor use before the procedure.

A total of 135 patients received a loading dose of clopidogrel (300 mg [$n = 75$], 600 mg [$n = 60$]) in the catheterization laboratory immediately after successful stenting. Patients on a maintenance dose of clopidogrel at the time of admission ($n = 57$) did not receive a loading dose. A GP IIb/IIIa inhibitor (eptifibatide, $n = 92$) was administered at the discretion of the treating physician. Patients not treated with GP IIb/IIIa inhibitors received unfractionated heparin to achieve an activated clotting time

≈ 300 s, and patients treated with a GP IIb/IIIa inhibitor achieved an activated clotting time of 200 s to 250 s. Aspirin was administered at a 81- to 325-mg daily dose for seven days before the procedure, and 325 mg was administered on the day of the procedure and daily thereafter. The maintenance dose of clopidogrel was 75 mg daily.

Blood sampling. Pretreatment blood samples were obtained in the catheterization laboratory before GP IIb/IIIa inhibitor or heparin administration through the indwelling femoral vessel sheath and transferred to vacutainer blood collecting tubes (Becton-Dickinson, Franklin Lakes, New Jersey) containing 3.8% trisodium citrate (for light transmittance aggregometry [LTA]) or 40 USP lithium heparin (for TEG assay) after discarding the first 2 to 3 ml of free flowing blood. The vacutainer tube was filled to capacity and gently inverted three to five times to ensure complete mixing of the anticoagulant. Blood samples were obtained at least 18 h after cessation of therapy in patients treated with a GP IIb/IIIa inhibitor. In the remaining patients, the discharge blood samples were obtained at least 24 h postprocedure.

LTA. Platelet aggregation was assessed as described previously (13). Briefly, the blood-citrate tubes were centrifuged at 120 g for 5 min to recover platelet-rich plasma (PRP) and further centrifuged at 850 g for 10 min to recover platelet-poor plasma (PPP). The PRP and PPP were stored at room temperature to be used within 2 h. Platelets were stimulated with 20 μM ADP, and the aggregation was assessed using a Chronolog Lumi-Aggregometer (Model 490-4D) with the Aggro/Link software package (Chronolog, Havertown, Pennsylvania). Aggregation was expressed as the maximum percent change in light transmittance from baseline, using PPP as a reference.

MA and fibrin generation time. The TEG Hemostasis Analyzer with automated analytical software provides quantitative and qualitative measurements of the physical properties of a clot (9-11). In essence, the TEG is a viscoelastic monitor that measures the degree of platelet-fibrin-mediated MA. Fibrin strands in the blood sample link a rotating sample cup with a stationary pin suspended by a torsion wire (Fig. 1). The

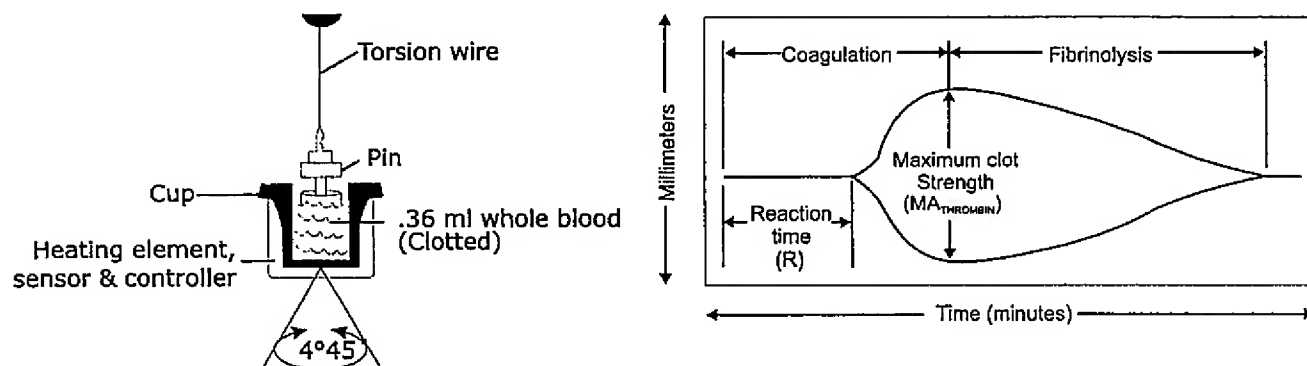


Figure 1. Schematic of thrombelastograph system: a torsion wire suspending a pin that is immersed in blood. As the clot forms while the cup is rotated 45°, the pin will rotate depending on the strength of the fibrin-platelet bonds. Signal is discharged continuously that reflects the onset of clotting (reaction time [R]) and the clot strength (MA).

Table 1. Patient Demographics

	Patients With Ischemic Events (n = 38)	Patients Without Ischemic Events (n = 154)	P Value
Age (yrs)	59 ± 10	62 ± 12	NS
Race (Caucasian) (%)	68	57	NS
Gender (male) (%)	42	60	0.05
BMI (kg/m ²)	31 ± 7	30 ± 7	NS
Risk factors/past medical history (%)			
Smoking	39	45	NS
Family history of CAD	47	32	NS
Hypertension	81	63	0.04
Hyperlipidemia	92	57	0.001
Diabetes	50	40	NS
Prior myocardial infarction	24	40	NS
Prior CABG	18	26	NS
Prior PTCA	42	39	NS
Pretreatment medications (%)			
Beta-blockers	90	81	NS
ACE inhibitors	74	61	NS
Calcium-channel blockers	21	22	NS
Lipid-lowering agents			
3A4 pathway metabolized	74	61	NS
Non-3A4 pathway metabolized	18	24	NS
Laboratory data			
WBC (× 1,000/mm ³)	7.3 ± 2.3	7.6 ± 2.4	NS
Platelets (× 1,000/mm ³)	244 ± 79	222 ± 66	NS
Hemoglobin (g/dl)	12.7 ± 2.3	13.3 ± 1.8	NS
Creatinine (g/dl)	1.1 ± 0.6	1.1 ± 0.8	NS

ACE = angiotensin-converting enzyme; BMI = body mass index; CABG = coronary artery bypass graft surgery; CAD = coronary artery disease; PTCA = percutaneous coronary angioplasty; WBC = white blood cells; 3A4 = hepatic cytochrome 3A4.

torque of the rotating cup is transmitted to the immersed pin. Pin movement is converted to an electrical signal by a transducer and is interpreted by the computer to create a tracing. The degree of platelet contribution to the MA through platelet-fibrin bonding directly influences the magnitude of pin

movement and ultimately the amplitude of the tracing. In the present study, the maximum amplitude of the thrombin-generated clot (MA) (mm) and the time from the start of the sample run to the first significant levels of clot formation (reaction time [R]) (min) were measured (Fig.

Table 2. Procedural Characteristics

	Patients With Ischemic Events (n = 38)	Patients Without Ischemic Events (n = 154)	P Value
Length of procedure (min)	55 ± 22	62 ± 34	NS
Ejection fraction (%)	48 ± 9	52 ± 9	NS
Number of vessels treated	1.3 ± 0.5	1.3 ± 0.6	NS
Lesion morphology			
De novo (%)	87	89	NS
Culprit lesion location (%)			
LAD	40	38	NS
CX	21	25	NS
RCA	34	30	NS
SVG	5	7	NS
Stent types (%)			
Drug-eluting	75	68	NS
Bare-metal	18	29	NS
PTCA only	7	3	NS
Reference vessel diameter (mm)	3.0 ± 0.4	3.0 ± 0.5	NS
Total lesion length (mm)	21.9 ± 10.1	19.0 ± 12.2	NS
Prestenosis (%)	86	84	NS
Poststenosis (%)	5	5	NS
Procedural success (%)	95	96	NS

CX = circumflex artery; LAD = left anterior descending artery; PTCA = percutaneous transluminal coronary angioplasty; RCA = right coronary artery; SVG = saphenous vein graft.

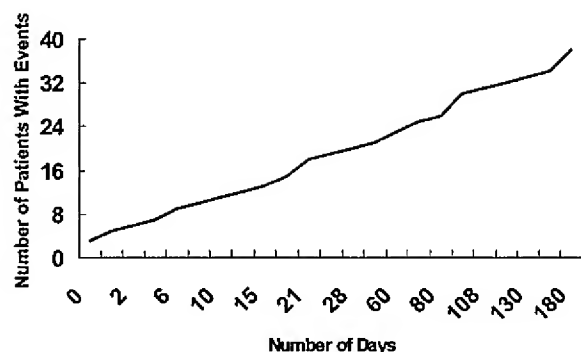


Figure 2. Graph demonstrating the time of occurrence of the first ischemic event.

1). The R parameter, a measure of initial thrombin-generated fibrin formation, has been correlated with the velocity of thrombin generation (9).

Blood was analyzed according to the manufacturer's instructions; 1 ml of heparinized blood was transferred to a vial containing kaolin and mixed by inversion; 500 μ l of the activated blood was then transferred to a vial containing heparinase and mixed to neutralize the heparin. The neutralized blood (360 μ l) was immediately added to a heparinase-coated cup and assayed in the TEG analyzer according to the manufacturer's instructions to obtain the thrombin-induced clot.

Definitions and clinical outcomes. Patients were contacted by telephone at the end of one month and six months to determine the occurrence of adverse events. Ischemic events were defined as the occurrence of death secondary to cardiovascular cause, myocardial infarction, unstable angina, and stroke that required rehospitalization. Myocardial infarction was defined as the occurrence of ischemic symptoms and a troponin I value greater than upper limits of normal. Unstable angina was defined as the occurrence of ischemic symptoms requiring rehospitalization. A physician blinded to the study results of the patient diagnosed all end points. Patients were divided into two groups based on the occurrence of adverse ischemic events. High LTA and MA were defined as >75th percentile. Low R was defined as <25th percentile.

Statistical analysis. The linear logistic regression model was employed to fit the binary data (ischemic event = 1 and nonischemic event = 0) while comparing different quartiles.

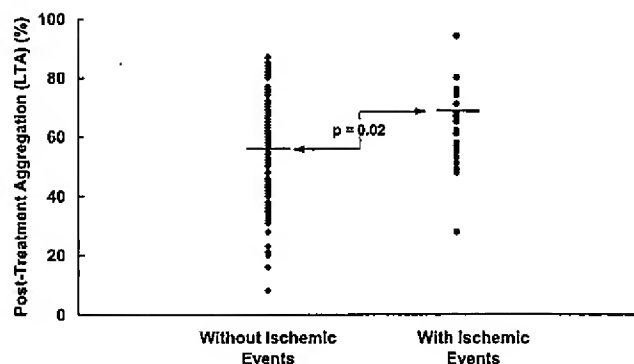


Figure 3. Adenosine-diphosphate-induced posttreatment platelet aggregation (20 μ M) measured by light transmittance aggregometry (LTA) in patients without ischemic events and with ischemic events.

This logistic regression model was fit using SAS procedure PROC LOGISTIC (SAS Inc., Cary, North Carolina). The model is given by:

$$\text{Logit}(p) = \text{Log}\{p / (1 - p)\} = \beta_0 + \beta_1 \cdot \text{QUARTILE_X}$$

where p = proportion of incidence of ischemic event, and QUARTILE_X is a factor with four quartiles of the variable X as its four levels. Appropriate pairwise comparisons were made using the corresponding contrasts to assess the difference between the two levels of the factor with respect to the proportion of ischemic events. The three variables (possible X) considered here are MA, LTA, and R.

The multiple linear logistic regression model was employed to fit binary data to compare the occurrence of difference risk factors in patients with ischemic events and without ischemic events. The logistic regression model was fit using SAS procedure PROC LOGISTIC (SAS Inc.). Odds ratio were calculated using SAS software, and receiver operator curves (ROC) were generated using MedCalc Software (Mariakerke, Belgium). Based on the normal distribution of data, the mean \pm SD is reported except as otherwise noted, and $p < 0.05$ was considered significant.

RESULTS

Patients and clinical outcomes. A total of 192 patients underwent catheter-based treatment and were analyzed. All of the procedures performed were nonemergent; 36 patients

Table 3. Evaluation of Platelet Function Tests by Light Transmittance Aggregometry and TEG

	Patients With Ischemic Events	Patients Without Ischemic Events	p Value
20 μ M ADP-induced pretreatment aggregation (%)	71 \pm 9	73 \pm 12	NS
20 μ M ADP-induced post-treatment aggregation (%)	63 \pm 12	56 \pm 16	0.02
TEG MA pretreatment (mm)	72 \pm 7	67 \pm 8	<0.001
TEG MA post-treatment (mm)	74 \pm 5	65 \pm 4	<0.001
Reaction time pretreatment (min)	4.6 \pm 2.3	4.7 \pm 2.0	NS
Reaction time post-treatment (min)	4.3 \pm 1.3	5.9 \pm 1.5	<0.001

ADP = adenosine diphosphate; MA = clot strength; TEG = thrombelastograph.

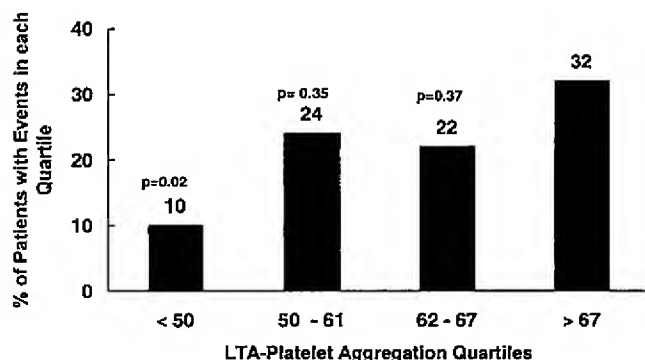


Figure 4. The observed frequency of patients with ischemic events in each quartile of light transmittance aggregometry (LTA) values is shown in the figure. The p values given in the figure indicate that the proportions of ischemic events in the first and the fourth quartiles are significantly different, whereas the proportions of ischemic events in the fourth quartile are not significantly different from the second or the third quartiles.

were admitted with unstable angina, and 11 patients had non-ST-segment elevation myocardial infarction. The remainder of the patients had stable angina. The patient demographics and procedural characteristics of patients with and without ischemic events are shown in Tables 1 and 2, respectively. There were four in-hospital ischemic events. All of these patients had myocardial infarction diagnosed by the occurrence of chest pain and increase in troponin I greater than upper limits normal. One of these patients had stent thrombosis.

Six-month follow-up data were complete in 191 of 192 patients. There were 44 events that occurred in 38 patients (20%) within six months of discharge (Fig. 2). All events occurred during aspirin therapy. Thirty-two patients were receiving aspirin and clopidogrel therapy at the time of their first event. Within one month after discharge, 20 of 191 patients (~10%) had events: myocardial infarction involving target vessel (n = 2), ischemia requiring revascularization of the prior target vessel (TVR) (n = 2 patients), ischemia involving a vessel other than the prior target vessel requiring revascularization (non-TVR) (n = 6), ischemia requiring hospitalization but not revascularization (n = 9), and stroke (n = 1). Between one and six months, 18 patients had the first occurrence of an event: death (n = 2), ischemia

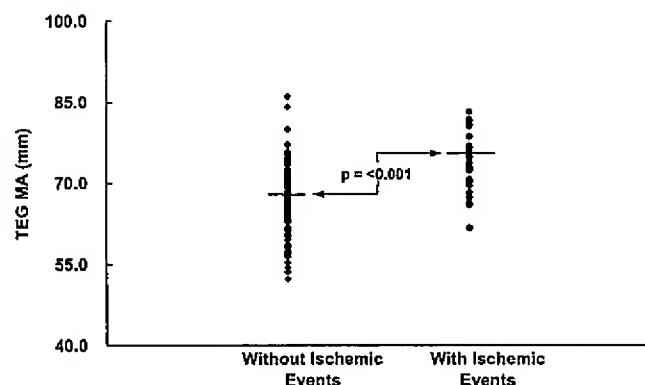


Figure 5. Post-treatment clot strength (MA) measured by thrombelastograph (TEG) in patients without ischemic events and with ischemic events.

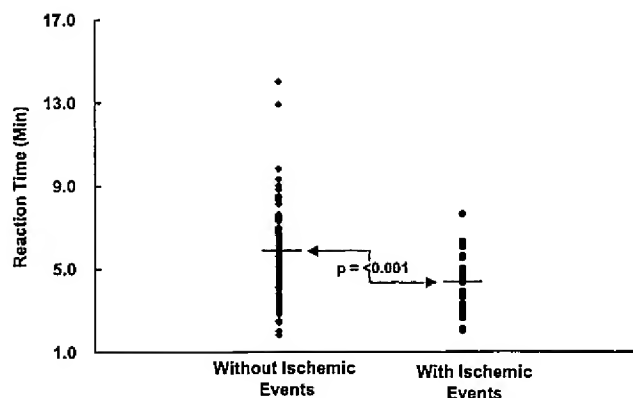


Figure 6. Post-treatment reaction time measured by thrombelastograph in patients without ischemic events and with ischemic events.

requiring TVR (n = 6), ischemia requiring non-TVR (n = 4), and ischemia requiring hospitalization but not revascularization (n = 6). Six patients had the occurrence of a second event between one and six months: coronary artery bypass grafting (n = 2), ischemia requiring TVR (n = 1), ischemia requiring non-TVR (n = 1), and ischemia requiring hospitalization but not revascularization (n = 2).

Platelet aggregation. A total of 160 patients had platelet aggregation measured by LTA, and 192 patients had blood samples analyzed by the TEG system (Table 3). Pretreatment aggregation by LTA was $71 \pm 9\%$ in patients with ischemic events and $73 \pm 12\%$ in patients without ischemic events (p = NS). Patients with ischemic events demonstrated a higher mean discharge ADP-induced platelet aggregation by LTA than patients without ischemic events (p = 0.02) (Table 3, Fig. 3). The change in mean platelet aggregation between pre- and postprocedure for the event group was 8% versus 17% for the group without events (p < 0.001). The greatest frequency of patients with ischemic events was present in the highest quartile of platelet aggregation (Fig. 4).

MA and R by TEG. Patients with ischemic events had significantly greater pre- and posttreatment MA than patients without ischemic events (p < 0.001) (Table 3, Fig. 5).

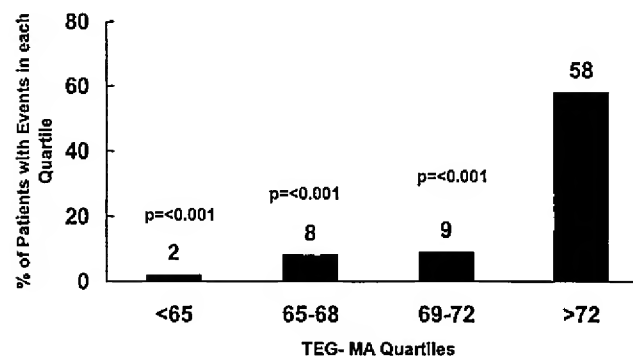


Figure 7. The observed frequency of patients with ischemic events in each quartile of clot strength (MA) values is shown in the figure. The p values given indicate that the proportion of ischemic events in each of the first three quartiles is significantly different from the proportion of ischemic events in the fourth quartile (p < 0.001). TEG = thrombelastograph.

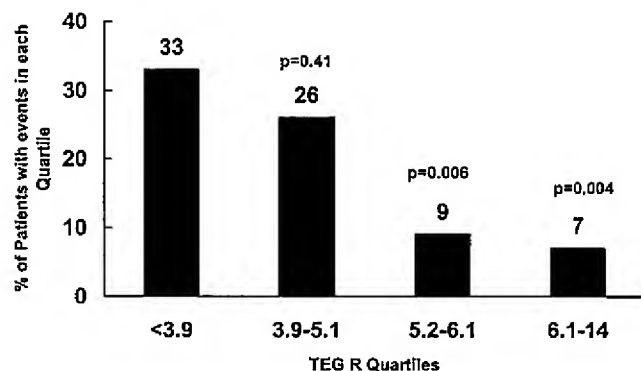


Figure 8. The observed frequency of patients with ischemic events in each quartile of reaction time (R) values is shown in the figure. The p values indicate that the proportions of ischemic events in first and the second quartiles are not significantly different ($p = 0.41$) whereas the proportions of ischemic events in the first quartile is significantly different from the third ($p = 0.006$) and the fourth quartiles ($p = 0.004$). TEG = thrombelastograph.

The R did not differ between groups at baseline but was significantly shorter in patients with events when measured postprocedure ($p < 0.001$) (Fig. 6). The highest quartile of MA and the lowest quartile of thrombin generation time were associated with the maximum occurrence of ischemic events (Figs. 7 and 8, respectively).

Patients with ischemic events. The occurrence of risk factors (high MA, low R, and high LTA) in patients with and without ischemic events, estimates of the parameters of the logistic regression model, odds ratios, and 95% confidence intervals are presented in Table 4. Among the 38 patients with events, only 4 patients had no risk factors at all (11%) as compared to 56% in the nonischemic group ($p < 0.0001$). The most prevalent risk factor in patients with ischemia was high MA (71%) with odds ratio estimate of 22.6 (95% confidence interval, 6.20 to 82.60), followed by a low R (42%, $p = 0.05$) with odds ratio estimate of 4.4 (95% confidence interval 1.00 to 19.05) and high LTA (35%, $p = 0.21$) with odds ratio estimate of 2.7 (95% confidence interval 0.56 to 12.96). Only 12% of patients without events demonstrated a high MA ($p < 0.0001$). The combined presence of two major risk factors, high MA and low R, in the ischemic events group was significantly higher (29%) compared to the non-ischemic events group (4%, $p < 0.0001$). The presence of all three risk factors was 100% predictive of an ischemic event.

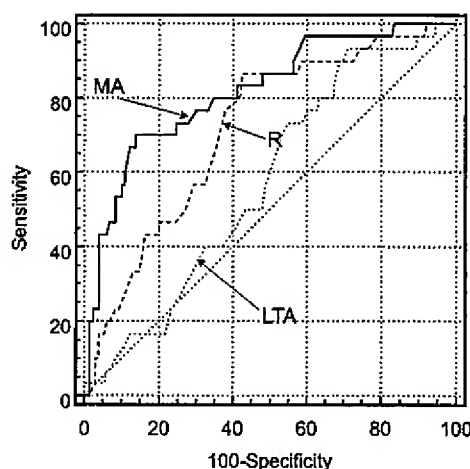


Figure 9. Combined receiver operator curve for clot strength (Thrombelastograph [TEG] MA), reaction time (TEG R), and 20 μ M adenosine-diphosphate-induced posttreatment platelet aggregation (light transmittance aggregometry [LTA]). High TEG MA has 74% sensitivity and 89% specificity; low TEG R has 42% sensitivity and 79% specificity; high LTA has 37% sensitivity and 79% specificity.

The combined ROC, sensitivity, and specificity for risk factors are shown in Figure 9. A high MA was the most specific and sensitive risk factor for the occurrence of ischemic events.

DISCUSSION

The current study suggests that in addition to high ex vivo platelet reactivity to ADP, greater overall MA and rapid fibrin generation are novel risk factors for the occurrence of post-PCI ischemic events. Greater reactivity to ADP in patients with ischemic events was supported by conventional LTA, the most common measurement reported in the literature. Thrombelastography has never been used as a predictive tool for ischemic events after PCI but has predictive value in surgical patients as a method to determine etiologies of bleeding (9,10). In the only other study that evaluated MA as a risk factor for thrombosis, McCrath et al. (11) demonstrated that noncardiac surgical patients with ischemic events had a significantly higher MA than those without events (~ 73 vs. ~ 65 mm, respectively). These data are concordant with our study.

A low incidence of ischemic events was observed in patients within the lowest quartile of aggregation measured

Table 4. Observed Frequency (%) of Patients Without Ischemic Events and With Ischemic Events in Risk Factor Groups, Estimates of the Parameters of the Logistic Regression Model, Odds Ratio, and 95% Confidence Intervals for the Odds Ratio

	High MA	Low R	High LTA	High MA and Low R	No Risk Factor
Patients without ischemic events, n (%)	18 (12%)	31 (21%)	30 (20%)	6 (4%)	83 (56%)
Patients with ischemic events, n (%)	27 (71%)	16 (42%)	13 (35%)	11 (29%)	4 (11%)
Estimate (SE)	3.12 (0.66)	1.47 (0.75)	0.99 (0.80)	3.64 (0.72)	—
p Value	<0.0001	0.0498	0.2129	<0.0001	—
Odds ratio estimate	22.6	4.4	2.7	38.0	—
95% confidence interval for the odds ratio	6.202–82.604	1.002–19.051	0.565–12.964	9.261–156.245	—

LTA = light transmittance aggregometry; MA = clot strength; R = reaction time.

by LTA, indicating the influence of successful P2Y₁₂ receptor blockade in the prevention of ischemic events. Indeed, the decrease in ADP-induced platelet aggregation levels after antiplatelet therapy was less pronounced in patients with events as compared to patients without events. The change in mean platelet aggregation between pre- and postprocedure for the event group was 8% versus 17% for the group without events ($p < 0.001$). The near absence of events in patients with <50% posttreatment aggregation induced by 20 μ M ADP may also suggest a therapeutic target for P2Y₁₂ inhibitors. More importantly, ~50% of the events occurred in patients with 25th to 75th percentile posttreatment platelet reactivity to ADP. This observation strongly suggests that agonists other than ADP play a dominant role in the genesis of ischemic events and that antiplatelet therapy directed against P2Y₁₂ and cyclooxygenase-1 in the current dosages is not sufficient to overcome thrombosis in selected patients.

In the current investigation, maximum MA measured by the TEG analyzer was the most sensitive and specific marker of ischemic risk and supports the central role of platelet reactivity to thrombin in recurrent ischemia post-PCI. A total of 58% of patients in the highest MA quartile developed events whereas those in the lowest two quartiles (lower quartiles) were nearly free of events. A total of 71% of the patients suffering from events were classified in the highest quartile of MA, and only 12% of patients without events were ranked in the highest quartile. Of these 12%, 76% had normal R as measured by the TEG analyzer. In ~40% of cases where the MA was high (>75th percentile), and an event occurred, the R was low (<25th percentile). When high MA was accompanied by a low R, there was an extremely high occurrence of ischemic events (odds ratio 38.0, $p < 0.0001$). These findings suggest that rapid thrombin generation often accompanies high MA. All of our data indicate that high responsiveness to thrombin (high MA) and accelerated thrombin generation (low R) are important predictive ex vivo measurements. Thus, selected patients with average ADP-induced posttreatment aggregation but with high MA and/or low R remain at risk for events.

Finally, the R significantly increased after PCI in those patients without ischemic events whereas in patients with events the mean value decreased. These findings suggest that effective clopidogrel therapy may influence thrombin generation. It has been reported that clopidogrel reduces thrombin generation (14,15).

Study limitations. Patients were not stratified before clinical events to different degrees of platelet reactivity or MA. It is uncertain whether improvements in the degree of platelet reactivity or MA before and after the procedure would help an individual patient in reducing their risk of a future ischemic event.

Conclusions. High ex vivo platelet reactivity and rapid generation of fibrin are risk factors for the development of ischemic events within six months of PCI. Moreover, MA,

a marker of thrombin-induced platelet-fibrin aggregation, is more predictive than platelet reactivity to ADP. These findings may explain the occurrence of events despite treatment with cyclooxygenase-1 and P2Y₁₂ inhibitors. Furthermore, it suggests the need to address effective inhibition of thrombin during and after PCI. Larger scale clinical trials are warranted to evaluate these prognostic measures and their implementation for therapeutic decisions.

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Platelet Reactivity in Patients and Recurrent Events Post-Stenting: Results of the PREPARE POST-STENTING Study

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Exhibit 6

Consensus and Future Directions on the Definition of High On-Treatment Platelet Reactivity to Adenosine Diphosphate

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The addition of clopidogrel to aspirin treatment reduces ischemic events in a wide range of patients with cardiovascular disease. However, recurrent ischemic event occurrence during dual antiplatelet therapy, including stent thrombosis, remains a major concern. Platelet function measurements during clopidogrel treatment demonstrated a variable and overall modest level of P2Y₁₂ inhibition. High on-treatment platelet reactivity to adenosine diphosphate (ADP) was observed in selected patients. Multiple studies have now demonstrated a clear association between high on-treatment platelet reactivity to ADP measured by multiple methods and adverse clinical event occurrence. However, the routine measurement of platelet reactivity has not been widely implemented and recommended in the guidelines. Reasons for the latter include: 1) a lack of consensus on the optimal method to quantify high on-treatment platelet reactivity and the cutoff value associated with clinical risk; and 2) limited data to support that alteration of therapy based on platelet function measurements actually improves outcomes. This review provides a consensus opinion on the definition of high on-treatment platelet reactivity to ADP based on various methods reported in the literature and proposes how this measurement may be used in the future care of patients.

Platelet activation and aggregation play pivotal pathophysiological roles in the development of ischemic events during and after acute coronary syndromes (ACS) and percutaneous coronary interventions (PCIs) (1). Adenosine diphosphate (ADP) is a major secondary agonist released from the dense granules of platelets activated by primary agonists (Fig. 1). The ADP-P2Y₁₂ receptor interaction plays a central role in the sustained activation of glycoprotein (GP) IIb/IIIa receptors leading to stable platelet-rich thrombus generation at the site of vessel wall injury (2). Therefore, clopidogrel, whose active metabolite irreversibly inhibits the P2Y₁₂ receptor, is a cornerstone of oral antiplatelet therapy in the secondary prevention of coronary artery disease and in the immediate treatment of ACS and PCI (3).

A significant reduction in ischemic complications in a wide range of coronary artery disease patients has been demonstrated in major randomized controlled trials by adding clopidogrel to aspirin treatment (4,5). The fixed dose, “one size fits all” treatment strategy with clopidogrel therapy, which has been used in clinical trials and recommended by current guidelines, does not take into account the interindividual pharmacodynamic variability of clopidogrel therapy (4–6). Moreover, despite the relatively potent antiplatelet effect of clopidogrel in some patients, others will suffer therapeutic failure manifested by ischemic events, including stent thrombosis, that have been associated with high on-treatment platelet reactivity (7).

These observations have stimulated intensive research of the pharmacodynamic and pharmacokinetic properties of

clopidogrel. Studies measuring platelet function in patients administered clopidogrel revealed that, unlike aspirin and GP IIb/IIIa receptor blocker therapies that are associated with a uniform and high level of inhibition (~95%) of their targets (COX-1 enzyme and GP IIb/IIIa receptor, respectively) with appropriate dosing in particular for GP IIb/IIIa inhibitors, clopidogrel treatment is associated with an overall variable and modest level of P2Y₁₂ inhibition even when high loading doses are used (4,6,8–10). In addition to distinct response variability, a substantial percentage of patients will also exhibit complete nonresponsiveness (resistance) to clopidogrel (10).

Multiple studies now have demonstrated a relationship between clopidogrel nonresponsiveness and/or high on-treatment platelet reactivity measured by multiple platelet assays and adverse clinical ischemic events (7). However, due to a lack of consensus on the optimal methods to quantify high platelet reactivity and the cutoff values associated with clinical risk, the routine measurement of platelet reactivity has not been widely implemented in clinical practice nor recommended in the guidelines (11). In addition, there are only limited data to support the concept that alterations of therapy based on platelet function measurements improve clinical outcome (7).

Herein, we provide a comprehensive overview of the available data that have identified high on-treatment platelet reactivity to ADP as a risk factor for post-PCI ischemic/thrombotic events as well as a consensus opinion on the definition of high on-treatment platelet reactivity to ADP based on the primary methods reported in the literature.

Clopidogrel Metabolism

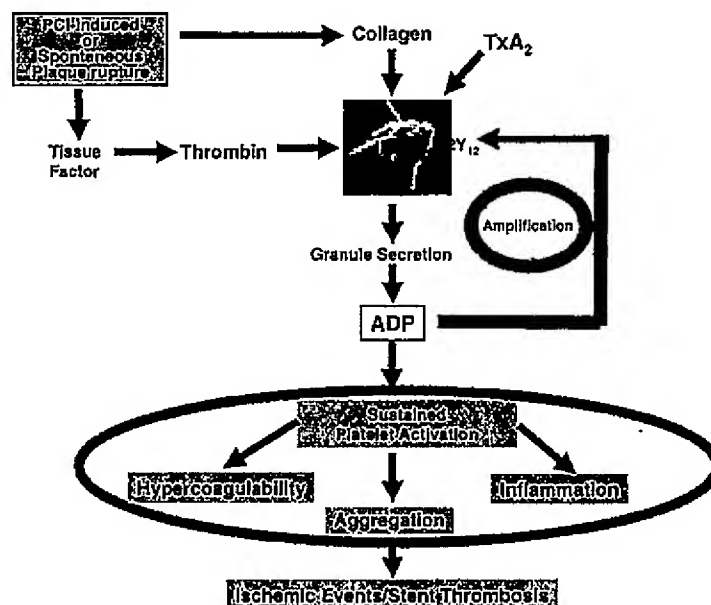
Clopidogrel is a prodrug that requires hepatic conversion into an active metabolite to exert its antiplatelet response. Most of absorbed clopidogrel (~85% to 90%) is hydrolyzed by carboxylase to an inactive carboxylic acid metabolite, SR26334, whereas the remaining ~10% to 15% is rapidly metabolized by hepatic cytochrome (CYP) P450 isoenzymes in a 2-step process. In the first step, the thiophene ring of clopidogrel is oxidized to 2-oxo-clopidogrel, which is then hydrolyzed to a highly labile active metabolite, R-130964, that has both carboxylic acid and thiol groups (12–14). Recent studies indicate that CYP2C19, CYP1A2, and CYP2B6 participate in the first metabolic step, whereas CYP2C19, CYP2C9, CYP2B6, and CYP3A are responsible for the second step (12,13) (Fig. 2). The highly unstable active metabolite, R-130964, covalently binds to platelet P2Y₁₂ receptor specifically and irreversibly during passage through the hepatic circulation resulting in inhibition of ADP-induced platelet activation-aggregation for the life span of the platelet (15). This metabolic activation scheme

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Figure 1

Central Role of ADP-P2Y₁₂ Receptor Interaction in Platelet Activation and Aggregation



Central role of adenosine diphosphate P2Y₁₂ receptor interaction in platelet activation and aggregation during occurrence of ischemic events and stent thrombosis. After plaque rupture, tissue factor and collagen are exposed leading to platelet activation. Three important pathways (thrombin-PAR-1 receptor, thromboxane A₂-TP receptor, and adenosine diphosphate P2Y₁₂ receptor) amplify the response. The adenosine diphosphate P2Y₁₂ interaction plays a central role. ADP = adenosine diphosphate; PCI = percutaneous coronary intervention; TxA₂ = thromboxane A₂.

is consistent with the time-dependent cumulative inhibition of ADP-induced platelet aggregation as observed with repeated daily dosing of clopidogrel and is further highlighted by slow recovery of platelet function following drug withdrawal (4,16,17).

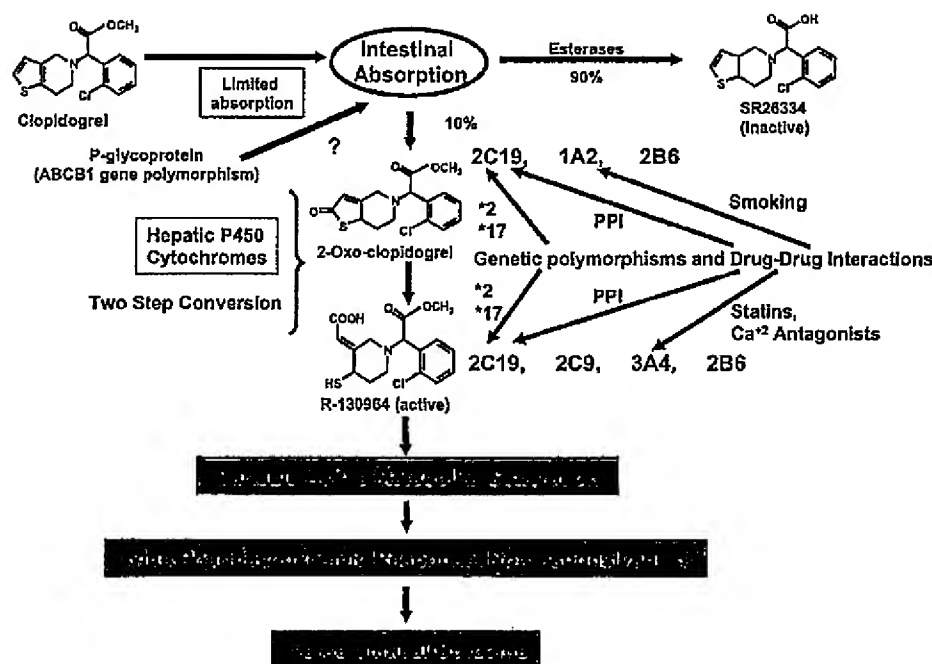
Multiple lines of evidence strongly suggest that variable and insufficient active metabolite generation are the primary explanations for clopidogrel response variability and nonresponsiveness, respectively (9). Variable levels of active metabolite generation following clopidogrel administration could be explained by: 1) variable or limited intestinal absorption, which may be affected by an *ABCB1* gene polymorphism (18–20); 2) functional variability in P450 isoenzyme activity influenced by drug-drug interactions as well as other factors; and 3) single nucleotide polymorphisms of specific genes encoding CYP450 isoenzymes (21,22). Stimulation of CYP3A4 activity by rifampin and St. John's wort and CYP1A2 activity by tobacco smoking have both been shown to enhance platelet inhibition induced by clopidogrel (23–25). The effect of smoking on the antiplatelet effect of clopidogrel has been associated with clinical outcomes and may, in part, explain the “smoker's

paradox” (26,27). Conversely, agents that compete with clopidogrel for CYP and/or inhibit CYP attenuate the antiplatelet effect of clopidogrel. A diminished pharmacodynamic response to clopidogrel has been observed with coadministration of proton pump inhibitors, lipophilic statins, and calcium-channel blockers that are metabolized by the CYP2C19 and CYP3A4 isoenzymes (21,28–31). Although a diminished level of platelet inhibition induced by clopidogrel has been demonstrated in some *ex vivo* studies following coadministration of these agents, the consequence of these interactions with respect to ischemic events remains controversial.

Recent studies have evaluated the influence of the single nucleotide polymorphisms of genes encoding CYP2C19 isoenzymes with different activities, as well as single nucleotide polymorphisms of the p-glycoprotein transporter gene on clopidogrel response variability and clinical outcomes (22,32). Multiple independent studies have demonstrated a link between the presence of genetic polymorphisms associated with suboptimal clopidogrel active metabolite generation (pharmacokinetic measurement), decreased clopidogrel responsiveness as measured by platelet function assays (pharmacodynamic measurement), and adverse clin-

Figure 2

Clopidogrel Response Variability



Clopidogrel response variability is a pharmacokinetic problem primarily influenced by the activity of cytochrome P450 isoenzymes in the generation of the active metabolite. Absorption may be affected by polymorphism of the *ABCB1* gene. The activity of hepatic cytochrome isoenzymes are influenced by drug-drug interactions, single nucleotide polymorphisms, and environmental influences (smoking).

ical outcomes. No single study has conclusively associated all of these parameters in the same patient population. Moreover it was observed that other genetic determinants may be involved and that overall, ~12% of the variation in the response to clopidogrel can be attributed to the *CYP2C19**2 loss-of-function allele (33). At this time, it is uncertain whether the factors associated with a poor response to clopidogrel are additive in diminishing the antiplatelet effect of clopidogrel and worsening patient outcomes.

The controversy surrounding the diminished effectiveness of clopidogrel in poor metabolizers (those having 2 loss-of-function *CYP2C19* alleles) and the utility of genetic tests to identify differences in *CYP2C19* function has been recently highlighted by the "boxed warning" issued by the Food and Drug Administration advising health care professionals to consider use of other antiplatelet medications or alternative dosing strategies for clopidogrel in these patients (34). The preceding statement was based on observations from a study of 40 healthy subjects that poor metabolizers had diminished active metabolite exposure and higher platelet aggregation. Although it is believed that the loss-of-function

allele confers its clinical risk by affecting the pharmacodynamic response to clopidogrel, no single study thus far has demonstrated a conclusive link between the presence of a loss-of-function genetic polymorphism, suboptimal clopidogrel active metabolite generation (pharmacokinetic measurement), decreased clopidogrel responsiveness (pharmacodynamic measurement), and adverse clinical outcomes. The warning only addresses patients with 2 loss-of-function alleles. No information is provided for heterozygotes. Earlier Simon et al. (18) suggested that increased ischemic risk is confined to homozygotes. Other studies involving patients treated with stenting found a significant relation between ischemic risk and loss-of-function allele carriers (homozygotes and heterozygotes) (33,35-38). The picture is even more confusing with the recently presented CHARISMA (Clopidogrel for High Atherothrombotic Risk, Ischemic Stabilization, Management, and Avoidance) Genomics substudy (39) results that showed an increase in the combined end point of cardiovascular death, myocardial infarction, and stroke in poor metabolizers (*2/*2) compared with wild-type carriers (wt/wt) treated with clopidogrel. Unlike the latter studies,

CHARISMA investigated a lower-risk population and was not a study of stented patients. The CHARISMA Genomics study investigators pointed out 2 important caveats: 1) poor metabolizers in the placebo arm also had an increased risk; but 2) only a small number of primary events occurred in poor metabolizers (placebo arm, $n = 5$ [8.77%] and clopidogrel arm, $n = 8$ [13.79%]). The CHARISMA Genomics study (39) is the only investigation in which the influence of genotyping on clinical outcome was studied in both the clopidogrel arm and the placebo arm.

Moreover, the safety and efficacy of altering therapy in response to genotype is entirely unknown. Whereas neither genotyping nor platelet function tests alone adequately describe the global risk profile of an individual patient treated with clopidogrel, point-of-care platelet function testing to identify high-risk patients combined with CYP2C19 genetic testing may be more effective in identifying high-risk individuals for alternative antiplatelet therapies. Ultimately, prospective randomized clinical trials will be needed to test specific personalized antiplatelet algorithms to provide the evidence base necessary for widespread adoption into clinical practice.

In addition to the preceding mechanisms for clopidogrel pharmacodynamic variability, increased body mass index, diabetes mellitus, and acute coronary syndromes have also been associated with a diminished antiplatelet response to clopidogrel (40–42). Several studies have demonstrated the coexistence of clopidogrel and aspirin resistance in the same patient population (43,44). It has also been demonstrated that patients with low responsiveness to a 600-mg loading dose, in addition to exhibiting a low level of inhibition of ADP-induced aggregation, also exhibit lower inhibition of aggregation induced by collagen and thrombin receptor agonist peptide as compared to moderate and high clopidogrel responders (45). Taken together, these data support the existence of a “hypo-responsive” or global high platelet-reactivity phenotype. Patients with the latter phenotype will have platelets that react robustly to multiple agonists. Finally, noncompliance is an obvious factor that must be excluded in the diagnosis of clopidogrel nonresponsiveness. When attempting to define causality for high platelet reactivity related to the occurrence of clinical events in patients receiving clopidogrel, all of the aforementioned mechanisms should be considered.

Concept of Clopidogrel Nonresponsiveness, Resistance, and High On-Treatment Platelet Reactivity

A single treatment strategy directed against a specific receptor cannot be expected to overcome all thrombotic events, and clinical treatment failure (occurrence of an

ischemic event) during clopidogrel treatment is not synonymous with clopidogrel resistance. The optimal definition of resistance or nonresponsiveness to any antiplatelet agent should be the failure of the antiplatelet agent to inhibit the target of its action (7). The identification of resistance should therefore utilize a laboratory technique that detects the activity of the target receptor before and after administration of the specific antiplatelet agent. For example, the absence of a change in platelet response (reactivity) to ADP from baseline after clopidogrel intake is an indicator of clopidogrel resistance. Earlier studies that measured light transmission platelet aggregation used an absolute difference of $\leq 10\%$ aggregation as the definition of clopidogrel resistance (baseline vs. on-treatment) (6,7). Patients were also categorized as “nonresponsive,” “semiresponsive,” and “responsive” using absolute platelet inhibition cut points of $<10\%$, 10% to 30%, and $>30\%$, respectively (6,46).

Even though a measurement of responsiveness (absolute or relative changes in platelet aggregation from baseline) appears as the most reliable indicator of a treatment effect, it may not be the optimal method to identify patients at high risk. Given the interindividual variability in baseline ADP-induced platelet aggregation, the measurement of clopidogrel responsiveness (inhibition) may overestimate ischemic risk in nonresponders with low pre-treatment reactivity as well as underestimate risk in responders who remain with high platelet reactivity after treatment (47,48). Therefore, the absolute level of platelet reactivity during treatment (i.e., on-treatment platelet reactivity) has been proposed as a better measure of thrombotic risk than responsiveness to clopidogrel.

The relationship of on-treatment platelet reactivity to both periprocedural and long-term ischemic risk has been most widely investigated. However, the optimal method to quantify platelet reactivity as well as the threshold definition for high on-treatment platelet reactivity to ADP have been subjects of controversy. Another concern surrounds the timing of platelet reactivity measurement that is optimally associated with short- and long-term risk. Any definition of high on-treatment platelet reactivity will only be meaningful when a cutoff or target value is identified by an accepted statistical test. Most commonly, the receiver-operator characteristic (ROC) curve analysis has been used to define the optimal cut point definition of high on-treatment platelet reactivity associated with ischemic risk. This method allows us to determine the cutoff value of platelet reactivity that would be associated with the lowest false negative and false positive rates and thus provides the greatest sum of sensitivity and specificity. The ROC curve analysis has been used to define cut points currently employed in prospective studies of individualized antiplatelet therapy in PCI patients (49).

Methods to Assess Platelet Responsiveness to ADP and P2Y₁₂ Receptor Reactivity

Because clopidogrel specifically inhibits the P2Y₁₂ receptor, *ex vivo* measurement of ADP-induced platelet aggregation in platelet-rich plasma by light transmittance aggregometry has been the most commonly used laboratory method to evaluate platelet inhibition by clopidogrel and its relation to ischemic risk. In the strictest sense, aggregometry evaluates an integrated response of the platelet to ADP through the function of both P2Y₁ and P2Y₁₂ receptors. In most studies, the maximal amplitude of measured platelet aggregation in response to 5-, 10-, or 20- μ mol/l ADP has been recorded. Citrate remains the most widely used anticoagulant during platelet function testing, although it affects intracellular calcium ion concentrations, which may influence platelet function. Alternatively, D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone or hirudin may be used to reduce changes in calcium ion concentrations. In addition to maximum platelet aggregation, late (final or residual) aggregation measured 5 to 6 min after the addition of agonist, a time when platelet disaggregation normally appears, has been proposed as a better indicator of clopidogrel responsiveness. Although Collet et al. (20) and Labarthe et al. (50) have correlated late aggregation with the antiplatelet response to clopidogrel, Gurbel et al. (51) suggested that clopidogrel nonresponders may be similarly identified by both maximal and late aggregation. Although some investigators have advocated the adjustment of platelet concentration in plasma to $\sim 250,000/\text{mm}^3$ before measuring, others have suggested that such an adjustment may introduce artifacts and contribute to assay variability (52). Unfortunately, because many other procedures involved in the performance of light transmittance aggregometry are not standardized between institutions, light transmittance aggregometry may not be the ideal test to monitor the effects of antiplatelet therapy outside of clinical trials (53).

Flow cytometric measurements of platelet expression of both activated GP IIb/IIIa receptor and P-selectin (CD62) following ADP stimulation in addition to ADP-induced platelet-fibrin clot strength as measured by whole blood thrombelastography have also been used to identify clopidogrel nonresponsiveness. Thrombelastography measurements correlated platelet function with ischemic risk in the PCI population (54,55). In addition, 2 point-of-care whole blood assays, the VerifyNow P2Y₁₂ assay (Accumetrics, San Diego, California) and the Multiplate analyzer (Dynabyte Informationssysteme, Munich, Germany) (both employing ADP as the agonist) have been used to measure platelet reactivity during clopidogrel therapy. The VerifyNow P2Y₁₂ assay is a turbidimetric assay that measures aggregation of platelets to fibrinogen-coated beads in whole blood. The

Multiplate analyzer is an impedance aggregometer that assesses platelet function in whole blood. The platelet function analyzer PFA-100 (Dade Behring, Deerfield, Illinois) method, which utilizes collagen/ADP-based cartridges and measures shear-induced platelet aggregation, has been associated with inconsistent estimates of platelet reactivity to ADP. Finally, the phosphorylation state of vasodilator-stimulated phosphoprotein (VASP) is a specific intracellular marker of residual P2Y₁₂ receptor reactivity in patients treated with P2Y₁₂ blockers, which is currently measured by flow cytometry and has also been correlated with ischemic risk (7). In addition, this is the only test that specifically assesses P2Y₁₂ receptor activity. Unlike methods employing the aggregation induced by ADP, in VASP, phosphorylation assay measurement does not include the contribution of the P2Y₁ receptor to the overall response (56).

Clopidogrel Nonresponsiveness and On-Treatment Platelet Reactivity: Early Studies

Järnmo et al. (57) first reported interindividual variability in response to clopidogrel in patients with coronary artery disease by using flow cytometry to detect ADP-induced fibrinogen binding to platelets. Gurbel et al. (6) first demonstrated clopidogrel response variability and resistance using conventional platelet aggregometry and flow cytometry studies in patients undergoing PCI who had received a 300-mg loading dose followed by 75-mg daily maintenance dose of clopidogrel. The level of platelet inhibition induced by clopidogrel was dependent on the time after clopidogrel treatment when platelet function was measured and the prevalence of resistance fell from 31% (days 1 and 5) to 15% (day 30). Importantly, although a 600-mg clopidogrel loading dose is associated with more potent platelet inhibitory effects than a 300-mg dose, this higher-dose regimen was not able to completely overcome resistance, and a broad variability in response profiles continued to persist (8,9). In the Gurbel et al. studies (6,8), pharmacologic resistance to clopidogrel was defined as an absolute $\leq 10\%$ decrease in platelet aggregation in response to agonist from baseline (pre-treatment measurement). Based on these studies, it became similarly apparent that the level of post-treatment platelet reactivity during clopidogrel therapy was largely unpredictable. Only early platelet reactivity (24 h after PCI) correlated with pre-treatment platelet reactivity (6).

Link Between High Platelet Reactivity and Post-PCI Ischemic/Thrombotic Events

Numerous studies have reported pharmacological “resistance” to clopidogrel as a potential etiology for thrombotic events after PCI (Table 1) (43,54–83). Barragan et al. (58)

were the first to demonstrate an association between post-treatment platelet reactivity and the occurrence of thrombotic events (clinical treatment failure) in a case-control study of PCI patients. In the study by Barragan et al. (58), a platelet reactivity index (PRI) >50% measured by VASP-phosphorylation assay was associated with thrombotic risk. Of note, in this study, turbidimetric aggregation was not associated with ischemic risk. However, at the same time, Matetzky et al. (59), using aggregometry, observed that patients undergoing primary PCI for ST-segment elevation myocardial infarction who were in the lowest quartile of clopidogrel responsiveness had the highest rates of ischemic events during follow-up.

Subsequently, it was suggested that the level of on-treatment platelet reactivity might be a superior risk predictor compared with the difference between baseline and post-treatment platelet reactivity, because platelet reactivity to ADP was variable before clopidogrel treatment in patients on aspirin therapy (47,48). The important relationship between high on-treatment platelet reactivity to ADP as measured by turbidimetric aggregometry and the occurrence of ischemic events in patients treated with stents was first prospectively demonstrated in the PREPARE POST-STENTING (Platelet Reactivity in Patients and Recurrent Events Post-Stenting) study (upper quartile, odds ratio: 2.6) (55). Multiple subsequent studies have confirmed the direct relationship between the level of platelet reactivity and post-PCI ischemic event occurrence using aggregation. Most recently, there have been further studies employing the VASP-phosphorylation assay, the VerifyNow P2Y₁₂ assay, and the Multiplate analyzer. These studies have consistently demonstrated that high on-treatment platelet reactivity is an important independent risk factor for the occurrence of thrombotic/ischemic events after PCI (56–84).

High Platelet Reactivity Defined by ROC Analysis

Importantly, studies have emerged that have used ROC curve analysis to define a threshold or cut point of on-treatment platelet reactivity associated with the optimal combination of sensitivity and specificity to identify thrombotic risk (Table 2). Thrombotic events may be prevented by achieving platelet reactivity below this threshold. It should be noted that such cut points might depend on the subset of patients studied. In fact, to date, cutoff values have been mainly investigated in patients undergoing PCI and different targets may be obtained in other settings depending on patient management or baseline risk profile (77,78).

Recent studies (62,64,72,76,77) have observed the prognostic value of the VASP phosphorylation analysis,

with an optimal cutoff value for VASP-PRI between 48% and 53%, which is similar to the threshold defined by Barragan et al. (58) in their earlier study of early stent thrombosis. Although these studies used different ischemic end points such as stent thrombosis or major adverse cardiac events (e.g., cardiovascular death, myocardial infarction, and urgent revascularization with or without stroke), they nevertheless found similar cutoff values for the VASP-PRI that were associated with post-PCI thrombotic event occurrence. Similarly, using the VerifyNow P2Y₁₂ assay, a cutoff value of ~240 P2Y₁₂ reaction units appears to be prognostic for subsequent thrombotic events (including cardiovascular death and stent thrombosis or cardiovascular death, nonfatal myocardial infarction, and stent thrombosis) (68,78,79,82). In a recent study, maximal platelet aggregation >46% in response to 5- μ mol/l ADP following PCI was associated with major adverse cardiac events (69). Using the Multiplate analyzer, Sibbing et al. (80) demonstrated that high on-treatment ADP-induced platelet reactivity measured before PCI was associated with the occurrence of 30-day stent thrombosis in 1,608 patients who had received a 600-mg clopidogrel loading dose before PCI. Moreover, based on ROC analysis, a cut point of 468 arbitrary aggregation units/min (approximately corresponding to the highest quintile) was associated with the occurrence of stent thrombosis (80). Recently, Breet et al. (82) evaluated the utility of multiple platelet function assays in predicting 1-year outcome of death, myocardial infarction, stent thrombosis, and stroke in 1,069 consecutive patients treated with clopidogrel following elective coronary stent implantation. In this large, prospective, observational study, high on-treatment platelet reactivity cut points of 42.9% maximal aggregation induced by 5- μ mol/l ADP and 64.5% by 20- μ mol/l ADP light transmittance aggregometry; 236 P2Y₁₂ reaction units measured by VerifyNow P2Y₁₂ assay; and 80.5% aggregation by Plateletworks (Helena Laboratories, Beaumont, Texas) all correlated with the occurrence of the composite primary end point, with an area under the curve of ~0.62 for each assay. The addition of high on-treatment platelet reactivity as measured by the noted platelet assays to more classical clinical and procedural risk factors improved the area under the curve to ~0.73 (82).

Each of these studies may thus provide a target level of platelet reactivity for future investigations, similar to the international normalized ratio used for warfarin therapy. The consistent findings across multiple investigations support the crucial role of high on-treatment reactivity in the etiology of ischemic events after PCI, including stent thrombosis, and suggest the existence of a threshold level of platelet reactivity below which ischemic events may be prevented

Table 1
Studies Linking High On-Treatment Platelet Reactivity to ADP and Clopidogrel Nonresponsiveness to Post-PCI Adverse Clinical Event Occurrence

Study (Ref. #)	Patients (n)	Treatment	Methods	Definition	Clinical Relevance
Barragan et al. (58)	PCI (48)	250 mg qd TLP or CLP 75 mg qd	VASP-PRI	>50% VASP-PRI	↑ ST
Gurbel et al. (55)	Elective PCI (192)	300-mg LD + 75 mg qd CLP +/- EPT	5-μmol/l ADP-LTA	HPR = 75th percentile post-PCI aggregation	↑ 6-month post-PCI events, OR: 2.7
Malatzky et al. (59)	PCI/STEMI (60)	300-mg LD + 75 mg qd CLP +/- EPT	5-μmol/l ADP-LTA	Reduction in platelet aggregation Upper quartile	↑ 6-month cardiac events
Gurbel et al. (60)	Elective PCI (120)	300-mg LD CLP +/- EPT	5-μmol/l ADP-LTA	Mean periprocedural platelet aggregation >50%	↑ Periprocedural myonecrosis
Gurbel et al. (61)	Elective PCI (200)	300-/600-mg LD CLP +/- EPT	5-μmol/l ADP-LTA	Mean periprocedural platelet aggregation >40%	↑ Periprocedural myonecrosis
Bliden et al. (54)	Elective PCI (100)	75 mg qd CLP	5-μmol/l ADP-LTA	>50% platelet aggregation	↑ 1-yr post-PCI events
Lev et al. (43)	Elective PCI (150)	300-mg CLP LD	5- and 20-μmol/l ADP-LTA	Baseline—post-treatment aggregation ≤10%	↑ Periprocedural myonecrosis
Blinda et al. (62)	High risk for ST/PCI (99)	75 mg qd for 6 months	VASP-PRI (72–96 h after stenting)	>48% PRI (ROC)	↑ 6-month ST
Cuisset et al. (63)	NSTEMI/ACS/PCI (190)	600-mg CLP LD >6 h before PCI	10-μmol/l ADP-LTA VASP-PRI	HPR >70% post-treatment LTA	↑ Periprocedural myonecrosis
Frere et al. (64)	NSTEMI/ACS/PCI (195)	600-mg CLP LD >6 h before PCI	10-μmol/l ADP-LTA	HPR (ROC) >70% post-treatment LTA >53% VASP-PRI	↑ 30-day post-PCI events MACE + stroke
Geisler et al. (65)	CAD/PCI (379)	600-mg CLP LD >6 h before PCI	20-μmol/l ADP-LTA	Clopidogrel low responders = <30% platelet inhibition	↑ 3-month MACE and death OR: 4.9
Geisler et al. (66)	CAD/PCI (1,092)	600-mg CLP LD >6 h before PCI + 75 mg qd	20-μmol/l ADP-LTA Residual aggregation measured after 5 min	Upper quartile	↑ 30-day MACE
Hochholzer et al. (67)	Elective PCI (802)	600-mg CLP LD >2 h before PCI + 75 mg qd	5-μmol/l ADP-LTA Residual aggregation measured after 5 min	Platelet aggregation above median	↑ 30-day MACE OR: 6.7
Price et al. (68)	PCI (380)	600-mg CLP LD >12 h before PCI or 75 mg qd >5 days	VerifyNow P2Y12 assay	HPR = post-treatment ≥235 PRU (ROC)	↑ 6-month post-PCI events including ST
Gurbel et al. (69)	Elective PCI (297)	300-/600-mg LD/ 75 mg qd CLP +/- EPT	5- and 20-μmol/l ADP-LTA	HPR = post-procedural (ROC) >46% 5-μmol/l ADP >59% 20-μmol/l ADP	↑ 2-yr Ischemic events 5-μmol/l ADP OR: 3.9 20-μmol/l ADP OR: 3.8
Gurbel et al. (70)	Stenting (120)	75-mg qd CLP >5 days	5- and 20-μmol/l ADP-LTA	HPR >75th percentile of platelet reactivity 5-μmol/l ADP = 50% 20-μmol/l ADP = 65%	↑ ST
Buonamici et al. (71)	PCI/DES (804)	600-mg LD + 75 mg qd for 6 months	10-μmol/l ADP-LTA	HPR ≥70% aggregation	↑ ST HR: 3.08
Bonello et al. (72)	PCI/stenting (144)	300-mg LD >24 h	VASP-PRI	>50% PRI (ROC)	↑ 6-month post-PCI MACE
Cuisset et al. (73)	PCI/SA (120)	600-mg LD ≥12 h before PCI	VerifyNow P2Y12 assay	↑ Platelet reactivity	↑ Post-PCI myonecrosis

continued on next page

Table 1

Continued

Study (Ref. #)	Patients (n)	Treatment	Methods	Definition	Clinical Relevance
Miglionini et al. (74)	PCI/DES/ULMD (215)	600-mg LD + 75 mg qd for 12 months	10- μ mol/l ADP-LTA	HPR \geq 70% aggregation	↑ 3-yr cardiac death and ST HR CV death: 3.82 HR ST: 3.69
Marcucci et al. (75)	PCI/ACS (683)	600-mg LD + 75 mg qd	VerifyNow P2Y ₁₂ assay	HPR \geq 240 PRU	12-month ischemic event HR CV death: 2.55 HR nonfatal MI: 3.36
Bonello et al. (76)	PCI/stenting (162)	600 mg repeated dose until PRI <50%	VASP-PRI	<50% VASP-PRI	↓ 1-month ischemic event
Bonello et al. (77)	PCI/stenting (214)	600-mg repeated dose until PRI <50%	VASP-PRI	<50% VASP-PRI	↓ Early ST and MACE (OR: 9.4)
Valgimigli et al. (78)	Elective PCI (1,277)	600-mg LD before PCI	VerifyNow aspirin and P2Y ₁₂ assay	>235 PRU >550 ARU	↑ Post-PCI myonecrosis
Patti et al. (79)	PCI (160)	600-mg LD or 75 mg qd >5 days	VerifyNow P2Y ₁₂ assay	HPR \geq 240 PRU (Pre-PCI)	↑ 1-month major cardiovascular event occurrence
Sibbing et al. (80)	PCI/DES (1,608)	600-mg LD before PCI	6.4- μ mol/l ADP Multiplate analyzer	Upper quintile (>416 AU/min) (ROC)	↑ 1-month definite ST (OR: 9.4)
Culsot et al. (81)	NSTEMI/stenting (598)	600-mg LD \geq 12 h before PCI	10- μ mol/l ADP-LTA VASP-PRI	>67% aggregation (ROC)	↑ ST
Breest et al. (82)	Elective PCI (1,069)	75-mg qd >5 days 300-mg LD >1 day 600-mg LD	20- μ mol/l ADP-LTA VerifyNow P2Y ₁₂ 20- μ mol/l ADP Plateletworks Before PCI	>42.9% 5- μ mol/l ADP (ROC) >64.5% 20- μ mol/l ADP >236 PRU 80.5% Plateletworks	OR for 1-yr death, MI, ST, and stroke 5- μ mol/l ADP: 2.09 20- μ mol/l ADP: 2.05 VerifyNow: 2.53 Plateletworks: 2.22

ACS = acute coronary syndromes; ADP = adenosine diphosphate; ARU = aspirin resistance units; AU = arbitrary aggregation units; CAD = coronary artery disease; CLP = clopidogrel; CV = cardiovascular; DES = drug-eluting stent; EPT = eptifibatide; HPR = high on-treatment platelet reactivity; HR = hazard ratio; LD = loading dose; LTA = light transmittance aggregometry; MACE = major adverse cardiac events; MI = myocardial infarction; NSTEMI = non-ST-segment elevated myocardial infarction; OR = odds ratio; PCI = percutaneous intervention; PRU = P2Y₁₂ reaction units; qd = once daily; ROC = receiver-operator characteristic curve; SA = stable angina; ST = stent thrombosis; STEMI = ST-segment elevated myocardial infarction; TLP = ticlopidine; ULMD = unprotected left main disease; VASP-PRI = vasodilator stimulated phosphoprotein—platelet reactivity index.

(62,64,68,69,72,75,80–82). Most importantly, the observed cut-off values for platelet reactivity noted previously had a very high negative predictive value for thrombotic/ischemic event occurrence, an observation of potential great clinical importance. However, the positive predictive value is fairly low for all assays. This is consistent with the fact that although it is a major determinant of thrombotic events, high on-treatment platelet reactivity is not the sole factor responsible for these events.

Personalized Antiplatelet Therapy: Preliminary Prospective Studies

Following the demonstration of a link between high on-treatment platelet reactivity in patients undergoing PCI together and thrombotic/ischemic events, several studies have aimed to lower the level of platelet reactivity by modifying therapy. These studies have demonstrated that platelet reactivity to ADP on standard clopidogrel therapy can be lowered by using higher loading or maintenance

doses of clopidogrel, the addition of cilostazol, switching to more potent alternative P2Y₁₂ receptor blockers such as prasugrel or ticagrelor (AstraZeneca, Wilmington, Delaware), and by adding elinogrel or GP IIb/IIIa inhibitors (76–78,85–93). An improved outcome with altered therapy was observed in some of these studies (76–78,93).

In 2 small multicenter trials that employed the VASP-phosphorylation assay, tailored incremental loading doses of clopidogrel further reduced on-treatment platelet reactivity below the previously noted threshold and were effective in reducing subsequent major adverse cardiac events without increasing Thrombolysis In Myocardial Infarction (TIMI) major or minor bleedings. However, it must be noted that about 5% of patients remain resistant to clopidogrel even after repeated loading doses of 600 mg (76,77). Similarly, following these findings, 2 other studies (82,91) have suggested that the selective administration of platelet GP IIb/IIIa receptor blockers to patients undergoing elective PCI who were identified as

Table 2

Studies Linking High On-Treatment Platelet Reactivity to Ischemic Events Based on ROC Curve With a Specific Cutoff Value

Study (Ref. #)	Assay	Cutoff Value	End Point	AUC	Odds Ratio
Price et al. (68)	VerifyNow P2Y12 assay	>235 PRU	6-month post-PCI CVD + MI + ST	0.71	NA
Gurbel et al. (69)	LTA	>46% 5-μmol/l ADP >59% 20-μmol/l ADP	2-year post-PCI MACE	0.77 0.78	3.9 3.8
Blindt et al. (62)	VASPPRI	>48% PRI	6-month ST	0.79	1.16
Frere et al. (64)	LTA VASPPRI	>70% 10-μmol/l ADP >53% PRI	1-month post-PCI MACE + stroke	0.74 0.73	NA
Bonello et al. (72)	VASPPRI	>50% PRI	6-month post-PCI MACE	0.55	NA
Marcucci et al. (75)	VerifyNow P2Y12 assay	≥240	1-yr CV death and nonfatal MI	0.66	2.38 CV death 2.76 nonfatal MI
Sibbing et al. (80)	Multipate analyzer-ADP	>468 AU/mln 6.4-μmol/l ADP	30-day ST	0.78	12.0
Culset et al. (81)	LTA	>67% 10-μmol/l ADP	1-month ST	0.69	5.8
Breet et al. (82)	LTA VerifyNow P2Y12 assay Plateletworks	>42.9% 5-μmol/l ADP >64.5% 20-μmol/l ADP >236 PRU >80.5% 20-μmol/l ADP	1-yr death, MI, ST, and stroke	0.63 0.62 0.62 0.61	2.09 2.05 2.53 2.22

AUC = area under the curve; CVD = cardiovascular disease; NA = not addressed; other abbreviations as in Table 1.

having high on-treatment platelet reactivity following an oral clopidogrel loading dose was effective in reducing subsequent post-PCI ischemic events without increased bleeding rates. These studies are the first to suggest that the cutoff value identifying patients at increased risk of thrombotic events could be used to tailor therapy and lead to an improved outcome.

Ongoing Studies of Personalized P2Y₁₂ Inhibitor Therapy

Larger clinical trials aimed at confirming the potential benefit of tailored doses of clopidogrel according to on-treatment platelet reactivity assessed by VerifyNow are currently recruit-

Table 3

Ongoing Clinical Studies Based Platelet Reactivity Measurement by VerifyNow Assay

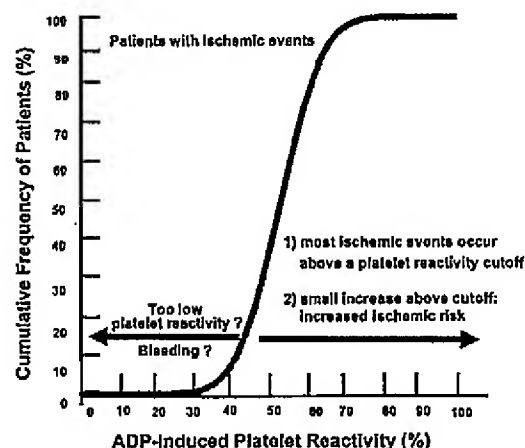
Study	ClinicalTrials.gov Identifier	Unstable or NSTEMI/PCI	Outcome	Clopidogrel Therapy
GRAMTAS Gauging Responsiveness With a VerifyNow Assay—Impact on Thrombosis and Safety	NCT00645918	Elective or ACS/PCI/DES (2,783)	6-month CV death, nonfatal MI, or ST	75 mg qd vs. 150 mg qd
ARCTIC Double Randomization of a Monitoring Adjusted Antiplatelet Treatment Versus a Common Antiplatelet Treatment for DES Implantation, and Interruption Versus Continuation of Double Antiplatelet Therapy	NCT00827411	Elective PCI/DES (2,500)	12-month composite end point of death, MI, stroke, urgent revascularization, ST	Therapy based on MD's performance
DANTE Dual Antiplatelet Therapy Tailored on the Extent of Platelet Inhibition	NCT00774475	Unstable or NSTEMI/PCI (442)	6- and 12-month CV death, nonfatal MI, TVR by PCI or CABG	75 mg qd vs. 150 mg qd
TOPAS-1 Tailoring of Platelet Inhibition to Avoid Stent Thrombosis	NCT00914368	Previous PCI or stenting for CAD (450)	6-month ST	600-mg LD 75 mg qd for 6 months
TRIGGER-PCI Testing Platelet Reactivity in Patients Undergoing Elective Stent Placement on Clopidogrel to Guide Alternative Therapy With Prasugrel	NCT00910299	PCI patients (2,150)	CV death, nonfatal MI	Prasugrel 60/10 mg vs. clopidogrel 600/75 mg

CABG = coronary artery bypass graft; MD = maintenance dose; TVR = target vessel revascularization; other abbreviations as in Table 1.

ing patients (Table 3) (94). The clinical benefit of achieving lower levels of on-treatment platelet reactivity was suggested by the TRITON-TIMI 38 (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel-Thrombolysis In Myocardial Infarction 38) (95) and the PLATO (Platelet Inhibition and Patient Outcomes) trials (96). In TRITON-TIMI 38, prasugrel, a third-generation thienopyridine associated with faster and lower on-treatment platelet reactivity than clopidogrel, was in turn associated with a lower prevalence of thrombotic events in ACS patients treated with PCI (95,97). However, prasugrel was associated with greater bleeding rates in the TRITON-TIMI 38 trial that may be related to excessively low platelet reactivity in selected patients (97). In the PLATO study, ticagrelor, the first oral nonthienopyridine reversible P2Y₁₂ inhibitor that provides a faster platelet inhibition and lower on-treatment platelet reactivity than clopidogrel was also associated with lower rates of ischemic events in an ACS population. Similar to the results of TRITON-TIMI 38, increased bleedings in ACS patients undergoing PCI were also noted in the ticagrelor group (95-97). These findings are consistent with the hypothesis that lower levels of platelet aggregation are associated with reduced ischemic events but increased bleeding risk. In the PLATO study, a similar bleeding event rate in patients undergoing coronary artery bypass grafting where ticagrelor therapy was discontinued within 3 days before surgery was observed (96). This was supported by the observation that ticagrelor was associated with faster offset of antiplatelet effects compared with clopidogrel therapy despite superior platelet inhibition in the ONSET/OFFSET (Randomized Double-Blind Assessment of the Onset and Offset of the Antiplatelet Effects of Ticagrelor Versus Clopidogrel in Patients With Stable Coronary Disease) study (17). Moreover, in the RESPOND (Response to Ticagrelor in Clopidogrel Nonresponders and Responders and the Effect of Switching Therapies) study (93), ticagrelor therapy was associated with uniform and superior platelet inhibition in both previously identified clopidogrel responders and non-responders, and that inhibition, in turn, was associated with an extremely low prevalence of high on-treatment platelet reactivity. In addition, another novel reversible P2Y₁₂ receptor blocker, clinogrel, has been shown to be associated with enhanced platelet inhibition when administered to selected patients with high platelet reactivity during standard clopidogrel therapy. Moreover, the antiplatelet effect of elinogrel was completely reversible within 24 h (92). The previously discussed alternative therapies may provide important advances to attenuated ischemic events occurrence, particularly in selected patients with high platelet reactivity on standard clopidogrel treatment. Dose adjustments based

Figure 3

Post-PCI Ischemic/Thrombotic Clinical Events



The sigmoid cumulative frequency curve in patients with post-percutaneous coronary intervention ischemic/thrombotic clinical events relative to platelet reactivity to adenosine diphosphate. These data support the concept of a therapeutic window for P2Y₁₂ blockade. Adapted, with permission, from Gurbel et al. (7). Abbreviation as in Figure 1.

on objective measurements of platelet reactivity may reduce the prevalence of bleeding. Reversibility may facilitate the management of patients requiring unanticipated surgery. The results of TRITON-TIMI 38 and PLATO suggest that there may be a fine balance between ischemic event occurrences and bleeding in patients treated with P2Y₁₂ receptor blockers. Consistently tailored P2Y₁₂ receptor blockade has the potential to improve outcome.

P2Y₁₂ Inhibitor Therapeutic Window

As platelet-mediated ischemic events appear to be clustered in the upper tertile or quartile of on-treatment platelet reactivity (i.e., above the optimal cut points previously identified), there may exist a "therapeutic window" for P2Y₁₂ receptor antagonist therapy that is associated with both an optimal reduction in thrombotic events as well as a low rate of major bleeding. The identification of a specific threshold for platelet reactivity that confers protection against thrombotic events and yet also limits bleeding following PCI is a crucial area of investigation, particularly in light of the increasing availability of platelet point-of-care assays as well as the widening choice of P2Y₁₂ receptor antagonists (7,60) (Fig. 3). At this time, there have been no definitive studies confirming a cut point of platelet reactivity to ADP associated with bleeding risk. However, recent observational data have emerged showing an association of an excessive response to clopidogrel and the occurrence of major

in-hospital bleeding events in clopidogrel-treated patients undergoing PCI (98–100). Moreover, the advent of more potent antiplatelet drugs that target the P2Y₁₂ receptor—such as prasugrel and ticagrelor, sets the need to study the relationship of antiplatelet treatment and risk for bleeding more thoroughly.

Future Considerations

It is unknown whether on-treatment platelet reactivity cut points associated with risk for periprocedural events are the same as those associated with long-term risk. Although similar cut points have been reported, the optimal platelet reactivity target may vary with respect to the time following the PCI procedure. For example, lower on-treatment platelet reactivity may be optimal in the early period following ACS and/or PCI, whereas the same low level may not provide the same clinical advantage 6 months later due to excessive bleeding. Also, the optimal level of platelet reactivity may differ between the settings of elective as compared to emergent PCI. Another factor that must be considered is that antiplatelet therapy responsiveness has been reported to improve over time following PCI, which may result in lower on-treatment platelet reactivity (6). Finally, the comparative utility of platelet function versus genetic testing should be investigated prospectively in order to determine whether these strategies are complementary or stand-alone methods to identify the high-risk patients.

Conclusions

The absolute level of platelet reactivity during treatment (i.e., on-treatment platelet reactivity) is proposed by the consensus of all the authors to be a better measure of thrombotic risk than responsiveness to clopidogrel. Currently available evidence supports the concept of a threshold for on-treatment platelet reactivity that may be used to stratify patient risk for ischemic/thrombotic events following PCI, including stent thrombosis. At the present time, high on-treatment platelet reactivity in the setting of PCI has been defined by ROC analyses using the following criteria: 1) PRI >50% by VASP-P analysis; 2) >235 to 240 P2Y₁₂ reaction units by VerifyNow P2Y₁₂ assay; 3) >46% maximal 5- μ mol/l ADP-induced aggregation; and 4) >468 arbitrary aggregation units/min in response to ADP by Multiplate analyzer (68,69,72,80) (Table 2). However, there are no large-scale clinical studies to date demonstrating that the adjustment of antiplatelet therapy based on any of these cut points improves clinical outcomes. Finally, PCI patients with diabetes and patients with ACS treated medically as compared to those treated with PCI may have different high on-treatment platelet reactivity cut points (84).

Ongoing studies with the VerifyNow P2Y₁₂ assay are underway to determine whether individually tailoring antiplatelet therapy will improve clinical outcomes after PCI. These studies will also investigate the relationship of platelet

reactivity to bleeding events. Currently, platelet function testing may be considered in determining an antiplatelet strategy in patients with a history of stent thrombosis and in patients prior to undergoing high-risk PCI. However, until the results of large-scale trials of personalized antiplatelet therapy are available, the routine use of platelet function measurements in the care of patients with cardiovascular disease cannot be recommended.

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99. Serebruany V, Rao SV, Silva MA, et al. Correlation of inhibition of platelet aggregation after clopidogrel with post discharge bleeding events: assessment by different bleeding classifications. *Eur Heart J* 2010;31:227–35.
100. Cuisset T, Cayla G, Frere C, et al. Predictive value of post-treatment platelet reactivity for occurrence of post-discharge bleeding after non-ST elevation acute coronary syndrome. Shifting from antiplatelet resistance to bleeding risk assessment? *Eurointervention* 2009;5:325–9.

Key Words: adenosine diphosphate ■ percutaneous coronary intervention ■ platelet reactivity ■ thrombotic events.

Exhibit 7

July 2011

Curriculum Vitae
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Professional Appointments

2004-present Director, Sinai Center for Thrombosis Research, Baltimore, MD

2009-present Interventional Cardiologist, Sinai Hospital of Baltimore

2007-present Sinai Hospital, Baltimore, MD
Associate Chief for Research of the Department of Medicine

2007-2009 Interventional Cardiologist, Hagerstown Heart, PA, Hagerstown, MD

2002-present President of Heartrials, LLC, Baltimore, MD

2001-2007 Partner and Interventional Cardiologist, Baltimore Heart Associates, PA, Baltimore, MD

2000-present President, Platelet and Thrombosis Research, LLC, Baltimore, MD

2000-2001 Interventional Cardiologist, Baltimore Heart Associates, PA, Baltimore, MD

1997-1999 Partner, Cardiovascular Specialists of Maryland, Baltimore, MD

1995-1997 Interventional Cardiologist, Heart Associates, PA, Baltimore, MD

Hospital Appointments

2007-present Sinai Hospital, Baltimore, MD
Associate Chief for Research of the Department of Medicine

2005-2006 Sinai Hospital of Baltimore
Helen Dalsheimer Director of the Division of Cardiology

2002-present Sinai Hospital, Baltimore, MD
Director of Therapeutics, Research and Technology Development for the Cardiac Catheterization Program

2001-present Lifebridge Health, Baltimore, MD
Director of Cardiovascular Research

2000-present Northwest Medical Center, Randallstown, MD,
Staff Physician

1999-present	Upper Chesapeake Medical Center, Bel Air, MD, Staff Physician
1998-present	Sinai Hospital, Baltimore, MD Director, Sinai Center for Thrombosis Research
1995-2000	Church Hospital, Baltimore, MD, Staff Physician Liberty Medical Center, Baltimore, MD, Staff Physician
1995-present	St. Joseph Medical Center, Towson, MD, Staff Physician Bon Secours Hospital, Baltimore, MD, Staff Physician
1995-present	Greater Baltimore Medical Center, Baltimore, MD, Staff Physician St. Agnes Hospital, Baltimore, MD, Staff Physician Union Memorial Hospital, Baltimore, MD, Staff Physician Sinai Hospital of Baltimore, Baltimore, MD, Staff Physician
1991-1995	University of Maryland Medical System, Baltimore, MD, Director of Interventional Cardiology
1990-1995	University of Maryland Medical System, Baltimore, MD, Medical Attending
1988-1990	Duke University Medical Center, Durham, NC, Medical Attending

Academic Appointments

2003-present	Johns Hopkins University School of Medicine, Baltimore, MD Associate Professor of Medicine (part-time)
1999-2003	Johns Hopkins University School of Medicine, Baltimore, MD Assistant Professor of Medicine (part-time)
1991-1995	University of Maryland School of Medicine, Baltimore, MD Director, Interventional Cardiology
1990-present	University of Maryland School of Medicine, Baltimore, MD Assistant Professor of Medicine
1990 - 1991	University of Maryland School of Medicine, Baltimore, MD Director, Research in Interventional Cardiology
1988 – 1990	Duke University School of Medicine, Durham, NC Associate in Medicine

Education

B.A.	Natural Sciences Johns Hopkins University, Baltimore, MD 1979
M.D.	University of Maryland School of Medicine, Baltimore, MD 1983

Post-Graduate Education

Internship	Internal Medicine Duke University Medical Center, Durham, NC
1983 – 1984	Residency – Internal Medicine Duke University Medical Center, Durham, NC
1984 - 1986	Assistant Chief Resident – Internal Medicine Duke University Medical Center, Durham, NC
1986- 1987	Fellowship - Pulmonary and Critical Care Johns Hopkins Hospital, Baltimore, MD

1986 – 1987 Fellowship – Cardiovascular Disease Duke University Medical Center, Durham, NC
 1987 – 1988 Chief Resident – Internal Medicine Duke University Medical Center, Durham, NC
 1988 – 1990 Fellowship – Interventional Cardiology Duke University Medical Center, Durham, NC

Awards and Honors

Elected Member of Phi Beta Kappa, Johns Hopkins University, 1979
 Elected Member of Alpha Omega Alpha, University of Maryland School of Medicine, 1983
 DuPont Pharmaceuticals, American College of Chest Physicians Young Investigator Award, 1991
 Faculty Research Award, Sinai Hospital of Baltimore, 2004 – 2005
 Daily Record Health Care Heroes Award, Advancement in Health Care, 2006

Board Certification

National Board of Medical Examiners, 1984
 American Board of Internal Medicine, 1986, Internal Medicine
 Board Eligible in Pulmonary and Critical Care Medicine, 1988
 American Board of Internal Medicine, 1989, Cardiovascular Disease
 American Board of Internal Medicine, 1999, 2010, Added Qualifications in Interventional Cardiology

Licensures and Registrations

State of Maryland, D34366

Professional Societies

Member, American College of Physicians, 1983 - 1995
 Member, American College of Chest Physicians, 1986 - 1991
 Member, American Thoracic Society, 1987 - 1995
 Member, Duke University Clinical Cardiology Society, 1990 - present
 Member, American Federation for Clinical Research, 1991 - 1994
 Fellow, Council on Clinical Cardiology, American Heart Association, 1992 - present
 Fellow, American College of Chest Physicians, 1992 - present
 Fellow, American College of Cardiology, 1992 – present
 Faculty, Society for Vascular Medicine and Biology, 2003-present

Patents

Gurbel PA and Anderson RD: Autoperfusion dilatation catheter. United States Patent #5295959 issued March 22, 1994.
 Serebruany VL, Gurbel PA, O'Connor CM. Methods of inhibiting platelet activation with selective serotonin reuptake inhibitors. United States Patent # 6245782 issued June 12, 2001.
 Dalesandro MR, Gurbel PA, Serebruany VL. Determining a treatment plan for patients undergoing thrombotic events by monitoring p-Selectin. United States Patent # 6230713 issued May 15, 2001.
 Gurbel PA. Assessment of cardiac health and thrombotic risk in a patient.
 United States Patent # 7381536 issued June 3, 2008.
 Gurbel Paul A: The detection of restenosis risk in patients receiving a stent by measuring the characteristics of blood clotting including the measurement of maximum thrombin-induced clot strength. Gurbel Paul A April 2007: WO 2007/044278
 Gurbel Paul A: Methods for diagnosis and treatment of thrombotic disorders mediated by cyp2c19*2. PORTOLA PHARMACEUTICALS January 2011: WO 2011/006169

Committees

Medical School/Academic Committees
 Duke University School of Medicine: Admissions Committee, Residency Program in Medicine, 1988 -1989
 University of Maryland School of Medicine: Pharmacy and Therapeutics Committee, 1991 - 1995
 Sinai Hospital of Baltimore/Lifebridge Health: Member, Institutional Review Board, 1998 - 2009

Sinai Hospital of Baltimore/Lifebridge Health: Member, Medical Executive Committee, 2004 – 2009
 Scientific Session Committee; Society for Cardiovascular Angiography and Intervention, 2004-2007
 Scientific Session Committee; Transcatheter Cardiovascular Therapeutics, 2004-present
 International Scientific Advisory Board; International Society of Thrombosis and Haemostasis, 2007-present

Other Committees

Member, Advisory Board, Datascope, Inc., 1992 - 1999
 Member, Advisory Board, Genentech, Inc., 1999 - 2001

Consultant /Advisory Board Appointments

Medtronic, Inc., 1992 - 1998
 Bristol Myers Squibb/Sanofi/Aventis, 1998 -present
 Dupont Merck Pharmaceuticals, 1998 - 2000
 Cordis Corp., 2001 – present
 Bayer Healthcare, 2003- present
 Astra Zeneca, 2003 - present
 Millennium Pharmaceuticals, 2002 – 2005
 Schering Plough, 2005-present
 Daiichi Sankyo, 2005-present
 Pozen Pharmaceuticals, 2007-present
 Portola Pharmaceuticals, 2008-2010
 Novartis, 2010-present
 Takeda, 2011-present
 Merck, 2009-present

Major Teaching Experience

Lecturer, Dept. of Pathology, University of Maryland School of Medicine, 1980
 Educated and trained medical residents and students at Duke University Medical Center as the Assistant Chief Resident, 1985
 Organized Medicine lecture series for medical students at Duke University School of Medicine, 1988
 Organized Medicine Grand Rounds at Duke University Medical Center, 1988-89
 Prepared biographies of the current and past “giants” of Duke Medicine to educate the students, house staff and faculty of landmark events in the history of the Duke Department of Medicine, 1988-89
 In charge of the education of the Duke Medicine house staff, 1988-89
 Instituted Chief of Medicine Rounds at Duke University, 1988
 In charge of the education of medical students on second year rotation and sub-internship on Medicine at Duke University, 1988-89
 Trained Interventional Cardiovascular fellows at Duke University, 1990
 Directed the Interventional Cardiology Research Laboratory at University of Maryland and educated all fellows and students conducting research in the laboratory, 1990-95
 Instituted fellowship training program in Interventional Cardiology at the University of Maryland School of Medicine, 1991
 Directed the education of all Interventional fellows at University of Maryland, 1991-95
 Lecturer, Department of Physiology, University of Maryland School of Medicine, 1990-95
 Lecturer, Department of Pathology, University of Maryland School of Medicine, 1991-96
 Lecturer at Medicine and Cardiology Grand Rounds, University of Maryland School of Medicine and Baltimore Veterans Hospital, 1990-95
 Education and training of Medicine house staff at University of Maryland, 1990-95
 Conduct Teaching Rounds in Coronary Care Unit at Union Memorial Hospital, 1995-99
 Educate Medicine Residents during Research Rotation in Thrombosis Laboratory at Union Memorial Hospital, 1996-97
 Conduct Teaching Rounds in Coronary Care Unit, Johns Hopkins University/Sinai Hospital Program in Internal Medicine, 1998-present
 Educate, train medical students, house staff conducting research in the Sinai Center for Thrombosis Research, 1998-present
 Participate in cardiology education program for third and fourth year Johns Hopkins University students at Sinai Hospital,

1998-present

Advise/precept Johns Hopkins University/Sinai residents intending to pursue careers in cardiology, 1998-present

Editorial Activities

Editorial Position Member, Editorial Board, Heartdrug, 2001 - 2002

Member, Editorial Board, Clinical Geriatrics, 2004 – 2007

Member, Editorial Board, Journal of Medicine, 2008-present

Editorial Consultant, Journal of the American College of Cardiology, 2010-present

Manuscript Reviewer

Arteriosclerosis, Thrombosis and Vascular Biology

American Heart Journal

American Journal of Cardiology

BioDrugs

Blood Coagulation and Fibrinolysis

Coronary Artery Disease

Circulation

Catheterization and Cardiovascular Interventions

European Heart Journal

Journal of the American College of Cardiology

Journal of Thrombosis and Haemostasis

Journal of the American Medical Association

Lancet

New England Journal of Medicine

Platelets

Thrombosis and Haemostasis

Major Research Interests

Thrombosis and Vascular Biology

Innovations in Catheter-Based Treatment of Vascular Disease

Treatment of Myocardial Infarction

Platelet Activation in Heart Disease

Fibrinolytic Therapy/Platelet Activation

Antiplatelet Therapy/Mechanisms and Clinical Effects of Drug Resistance

Novel Pathways of Platelet-Platelet and Platelet-Leukocyte Interactions

Personalized Antiplatelet Therapy

Publications

Peer-Reviewed Articles:

1. Haber R, Oddone E, **Gurbel PA** et al.. Acute pulmonary decompensation due to amphotericin B in the absence of granulocyte transfusions. *N Engl J Med* 315:836,1986.
2. Navetta F, Barber M. **Gurbel PA**, Moreadith R, Bernstein R, Hlatky MA, Coleman RE, Bashore TM. Myocardial ischemia in constrictive pericarditis. *Am Heart J* 116:1107, 1988.
3. **Gurbel PA**, Davidson CJ, Smith JE, Ohman EM, Stack RS. Selective infusion of thrombolytic therapy in the acute myocardial infarct related coronary artery as an alternative to rescue percutaneous angioplasty. *Am J Cardiol* 66:1021, 1990.
4. Hurwitz JL, Davidson CJ, **Gurbel PA**, Greenfield R, Kassell JH, Kanter RJ. AV nodal blockade via selective AV nodal artery injection in a human. *Am Heart J* 120:986, 1990.
5. Tapson VF, Davidson CJ, **Gurbel PA**, Sheikh KH, Kisslo KB, Stack RS. Rapid and accurate diagnosis of pulmonary emboli in a canine model using intravascular ultrasound imaging. *Chest* 100:1410, 1991.

6. Herzog WR, Atar D, **Gurbel PA**, Vogel RA, Schlossberg ML, Serebruany VL. Effect magnesium infusion on platelet aggregation in swine. *Magnesium Res* 4:349-353, 1993.
7. **Gurbel PA**, MacCird CS, Anderson RD, Scott HJ, Atar D, Mergner W, Herzog WR: A canine coronary artery thrombosis model for the evaluation of reperfusion strategies. *Cardiology* 84:108, 1994.
8. **Gurbel PA**, Anderson RD, MacCord CS, Scott HJ, Poulton J, Goddard J. Arterial diastolic pressure augmentation by intra-aortic balloon counterpulsation enhances the onset of coronary artery reperfusion by thrombolytic therapy. *Circulation* 89:361-265, 1994.
9. Tapson VF, **Gurbel PA**, Witty LA, Pieper KS, Stack RS. Pharmacomechanical thrombolysis of experimental pulmonary emboli: rapid low-dose intraembolic therapy. *Chest* 106:1668-1652, 1994.
10. Ohman EM, Marquis J-F, Ricci DR, Brown RG, Knudtson ML, Kerejakes DJ, Samaha JK, Margolis JR, Niderman AL, Dean LJ, **Gurbel PA**, Sketch M, for the perfusion balloon catheter study group. A randomized comparison of gradual prolonged versus standard primary balloon inflation on early and late outcome results of a multicenter clinical trial. *Circulation* 89:1118-1125, 1994.
11. Ohman EM, George BS, White CJ, Kern MJ, **Gurbel PA**, Freedman RJ, Lundergan C, Hartmann JR, Talley JD, Frey MJ, Taylor G, Leimberger JD, Owens PM, Lee KL, Stack RS, Califf RM, for Randomized IABP Study Group. The use of aortic counterpulsation to improve sustained coronary artery patency during acute myocardial infarction. Results of a randomized trial. *Circulation* 90:792-799, 1994.
12. The Epic Investigators. Evaluation of chimeric monoclonal antibody C7E2 fab fragment directed against the platelet glycoprotein II_b III_a receptor for preventing ischemic complications of high risk angioplasty. (EPIC) *N Engl J Med* 330:956-957, 1994.
13. **Gurbel PA**, Anderson RD, MacCord CS, Scott HJ, Serebruany VL, Herzog WR. Accelerated intravenous dosing of recombinant tissue plasminogen activator causes rapid, but unstable reperfusion in a canine model of acute myocardial infarction. *Coron Artery Dis* 5: 929-936, 1994.
14. **Gurbel PA**, Serebruany VL, Komjathy SF, Collins ME, Sane DC, Scott HJ, Schlossberg ML, Herzog WR. Regional and systemic platelet function is altered by myocardial ischemia-reperfusion. *J Thromb and Thrombolysis* 1: 187-194, 1995.
15. Serebruany VL, Herzog WR, **Gurbel PA**, Schlossberg ML, Scott HG, Vogel RA. NPC15669, an anti-inflammatory leucine derivative reduces *in vitro* platelet aggregability in both swine and human plasma. *J Thromb and Thrombolysis* 1: 171-178, 1995.
16. **Gurbel PA**. Functional characteristics of a new perfusion balloon; comparison with the Flowtrack 40 in a closed chest swine. *Cathet Cardiovasc Diagn* 36:377-378, 1995.
17. Herzog WR, **Gurbel PA**, Vogel RA, Schlossberg ML, Scott HJ, Schneider AI, Serebruany VL. Effects of NPC 15669 an anti-inflammatory leucine derivative on myocardial stunning and pre-conditioned infarction size in swine. *J Thromb and Thrombolysis* 1:163-170, 1995.
18. **Gurbel PA**, Connor CM. Zofenopril after anterior myocardial infarction. *N Engl J Med* 332:1715, 1995.
19. **Gurbel PA**, Midwall JA, Brodie BR. Angioplasty and adjunctive intra-aortic balloon pump counterpulsation: Current clinical considerations. *Today's Therapy Trends* 13:63-78, 1995.
20. Benitez RM, **Gurbel PA**, Chong H, Rajasingh MC. Penetrating atherosclerotic ulcer of the aortic arch resulting in extensive and fatal dissection. *Am Heart J*. 129:821-3, 1995.

21. Pimentel CX, Schreiter SW, **Gurbel PA**. The use of the Tracker® catheter as a guidewire support device in angioplasty of angulated and tortuous circumflex coronary arteries. *J Invas Cardiol* 7:66-71, 1995.
22. **Gurbel PA**. Angioplasty and adjunctive intra-aortic balloon pump counter-pulsation. *Cardiac Assists* 7:1-4, 1995.
23. Gurbel PA, Haber HL. Use of antithrombotics in clinical cardiology. Risks vs Benefits. *J Outcomes Management* 3:11, 1996.
24. Serebruany VL, Herzog WR, **Gurbel PA**. Serial changes of natural antithrombotics during myocardial ischemia-reperfusion in swine. Effects of magnesium, diltiazem and a novel Mac-1 inhibitor. *Blood Coag Fibrinolysis* 7:632-640, 1996.
25. Gurbel PA, Navetta FI, Bates ER, Muller DW, Tenaglia AN, Miller MJ, Muhstein B, Hermiller JB, Davidson CJ, Aguirre FV, Beauman GJ, Berdan LG, Leimberger JD, Boville EG, Christenson RH, Ohman EM. Lesion-directed administration of tissue plasminogen activator with intracoronary heparin in patients with unstable angina and coronary thrombus undergoing angioplasty. *Cathet Cardiovasc Diagn* 37:382-391, 1996.
26. Anderson RD, **Gurbel PA**. The effect of intra-aortic balloon counterpulsation on coronary blood flow velocity distal to coronary artery stenosis. *Cardiology* 87:306-312, 1996.
27. Serebruany VL, Herzog WR, Atamas SP, **Gurbel PA**, Paulsen G, Mortensen SA, Folkers K. Hemostatic changes after dietary coenzyme Q10 supplementation in swine. *J Cardiovasc Pharm* 28:175-181, 1996.
28. Gurbel PA, Serebruany VL, Komjathy SF, Collins ME, Bittar GD, Schlossberg ML, Mergner W. Pretreatment with an inhibitor of Mac-1 alters regional and systemic platelet function during ischemia - reperfusion in swine. *Pharmacology* 53:79-86, 1996.
29. Serebruany VL, Solomon SR, Edenbaum LR, Herzog WR, **Gurbel PA**. Mac-1 inhibitor affects certain hemostatic parameters during myocardial stunning in swine. *Pharmacology* 53: 87-96, 1996.
30. Serebruany VL, Schlossberg ML, Edenbaum LR, Herzog WR, **Gurbel PA**. Intracoronary magnesium and diltiazem affect to a similar extent certain hemostatic factors during acute myocardial infarction in swine. *Pharmacology*. 53:224-233, 1996.
31. Serebruany VL, Schlossberg ML, Edenbaum LR, Herzog WR, **Gurbel PA**. Hemostatic changes after early versus late intracoronary magnesium during acute myocardial infarction in swine. *J Cardiovasc Pharm* 28:817-823, 1996.
32. Serebruany VL, Herzog WR, Edenbaum LR, Shustov AR, **Gurbel PA**. Changes in the haemostatic profile during magnesium deficiency in swine. *Magnesium Res* 9:155-163, 1996.
33. Gurbel PA, Criado FJ, Cumutte EA, Patten P, Secada-Lovio J. Percutaneous revascularization of an extensively diseased saphenous vein bypass graft with a saphenous vein-covered Palmaz stent. *Cathet Cardiovasc Diagn* 40:75-78, 1997.
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35. Serebruany VL, Herzog WR, **Gurbel PA**. Serial changes of the plasma prostanoids during myocardial ischemia - reperfusion in swine. Effects of magnesium, diltiazem, and a novel Mac-1 inhibitor. *Prostaglandins Leukot Essent Fatty Acids* 56:135-142, 1997.
36. Gurbel PA, Anderson RD: A new concept in coronary angioplasty. Dilatation with a helical balloon which allows

simultaneous autoperfusion. *Cathet Cardiovasc Diagn* 40:109-116, 1997.

37. **Gurbel PA**, Anderson RD, Pells HO, van Boven AJ, den Heijer P. Coronary artery angioplasty with a helical autoperfusion catheter. *Cathet Cardiovasc Diagn* 40:179-185, 1997.
38. Serebruany VL, **Gurbel PA**, Ordonez JV, Herzog WR, Rohde M., Mortensen SA, Folkers K. Could coenzyme Q10 affect hemostasis by inhibiting platelet vitronectin (CD51/CD61) receptor? *Molec Aspects Med* 18:S189-S194, 1997.
39. **Gurbel PA**, Herzog WR, Vogel RA, Schlossberg ML, Edenbaum LR, Scott HJ, Serebruany VL. Short-term low dose intracoronary diltiazem administered at the onset of reperfusion reduces myocardial infarct size. *Intern J Cardiol* 59:21-27, 1997.
40. Serebruany VL, Solomon SR, Herzog WR, **Gurbel PA**. Bolus magnesium infusion in humans is associated with predominantly unfavorable changes in platelet aggregation and certain hemostatic factors. *Pharm Res* 35:17-22, 1997.
41. Serebruany VL, Schlossberg ML, Edenbaum LR, Herzog WR, **Gurbel PA**. Effects of intracoronary diltiazem on certain hemostatic parameters during acute myocardial infarction in swine. *Inter J. Cardiol* 61:21-29, 1997.
42. **Gurbel PA**, Serebruany VL. Myths and realities of P-Selectin plasma levels in patients with acute myocardial infarction. *Thromb Res* 88:343-344, 1997.
43. Serebruany VL, **Gurbel PA**, Shustov AR, Dalesandro M, Gumbs SI, Grabletz BS, Bahr RD, Ohman EM, Topol EJ. Depressed platelet status in a patient with hemorrhagic stroke following thrombolysis for acute myocardial infarction. *Stroke* 29:235-238, 1998.
44. **Gurbel PA**, Serebruany VL. Soluble vascular cell adhesion molecule and E-Selectin in patients with acute myocardial infarction treated with thrombolytic agents. *Am J Cardiol* 81:772-775, 1998.
45. Serebruany VL, Atar D, Dalesandro MR, O'Connor CM, **Gurbel PA**. Changes in hemostasis after parenteral magnesium in myocardial ischemia reperfusion: Animal studies to clinical trials. *Magnes Res* 11:37-42, 1998.
46. Serebruany VL, Bahr RD, O'Connor CM, Lowry DR, **Gurbel PA**. For the GUSTO III Platelet Substudy. Antecedent aspirin therapy inhibits baseline platelet activity in patients presenting with acute myocardial infarction. *Cardiology* 90:3742, 1998.
47. Serebruany VL, Solomon SR, Herzog WR, **Gurbel PA**. Plasma fibronectin during myocardial ischemia - reperfusion. Effects of magnesium, diltiazem and a novel Mac-1 inhibitor. *Am J. Hematol* 57:309-314, 1998.
48. Serebruany, VL, Solomon SR, Shustov AR, Herzog WR, **Gurbel PA**. Survival in acute myocardial infarction induced by coronary artery ligation: prognostic relevance of certain hemostatic factors during the occlusion phase. *J Thromb and Thrombolysis* 5: 39-35, 1998.
49. **Gurbel PA**, Shustov AR, Bahr RD, Carpo C, Ohman EM and Topol EJ for the GUSTO III Investigators. Effects of reteplase and alteplase on platelet aggregation and major receptor expression during the first 24 hours of acute myocardial infarction treatment. The GUSTO III Platelet Study. *J Am Coll Cardiol* 31:1466-1473, 1998.
50. Serebruany VL, Ordonez JV, Yrovsky VV, **Gurbel PA**. The crossreactivity of human vs swine platelet surface antigens is similar for glycoproteins II_b and III_a, but not for II_b and III_a complex. *J Thromb and Thrombolysis* 5:37-41, 1998.
51. Serebruany VL, **Gurbel PA**, Shustov AR, Ohman EM, Topol EJ. Heterogeneity of platelet aggregation and major surface receptor expression in patients presenting with acute myocardial infarction. *Am Heart J* 136:398-405, 1998.

52. Serebruany VL, **Gurbel PA**, Murugesan SR, Lowry Dr, Sturm E, Svetlov SI. Depressed plasma platelet-activating factor acetylhydrolase in patients presenting with acute myocardial infarction. *Cardiology* 90:127-130, 1998.
53. Serebruany VL, Schlossberg ML, Edenbaum LR, Herzog WR, **Gurbel PA**. Serial changes of soluble endothelin-1 levels during myocardial ischemia-reperfusion. Effects of magnesium, diltiazem and a novel Mac-1 Inhibitor. *Pharm Res* 38:165-172, 1998.
54. **Gurbel PA**, Serebruany VL, Shustov AR, Dalesandro M, Gumbs SI, Grabletz BS, Bahr RD, Ohman EM, Topol EJ. Increased baseline platelet P-selectin, and PECAM-1 as predictors of unsuccessful thrombolysis in patients with acute myocardial infarction. *Coron Artery Dis* 9:451-456, 1998.
55. **Gurbel PA**, Dalesandro MR, Serebruany VL. Reteplase but not alteplase affects early soluble PECAM-1 and P-selectin release in patients with acute myocardial infarction. *Thromb Haemost* 80:725, 1998.
56. Serebruany VL, **Gurbel PA**. Effect of thrombolytic therapy on the plasma concentration and platelet expression of the platelet/endothelial cell adhesion molecule in patients with acute myocardial infarction. *Arterioscl Thromb Vasc Biol* 19:153-158, 1999.
57. Serebruany VL, **Gurbel PA**. The relations of major platelet receptor expression during myocardial infarction. Monitoring efficacy of GP IIb/IIIa inhibitors by measuring P-selectin? *Thromb Haemost* 80:314-316, 1999.
58. Serebruany VL, **Gurbel PA**. Assessment of platelet activity by measuring platelet derived substances in plasma from patients with acute myocardial infarction: Surprising lessons from the GUSTO III Platelet Study. *Thromb Res* 93:149-150, 1999.
59. O'Connor CM, **Gurbel PA**, Serebruany VL. Usefulness of soluble and surface-bound P-selectin in detecting heightened platelet activity in patients with congestive heart failure. *Am J Cardiol* 83:1345-1349, 1999.
60. Serebruany VL, Murugesan SR, Pothula A, Semaan H, **Gurbel PA**. Soluble platelet/endothelial cellular adhesion molecule-1, but not P-selectin, nor osteonectin identify acute myocardial infarctions among patients with chest pain admitted to the Emergency Department. *Cardiology* 91:50-55, 1999.
61. **Gurbel PA**, O'Connor CM, Cummings CC, Serebruany VL. Clopidogrel: The future choice for preventing platelet activation during coronary stenting? (Review) *Pharm Res* 65:109-123, 1999.
62. Nair GV, **Gurbel PA**, O'Connor CM, Gattis WM, Murugesan SR, Serebruany VL. Depression, coronary events, platelet inhibition and serotonin reuptake inhibitors, (editorial). *Am J Cardiol* 84:321-323, 1999.
63. Serebruany VL, Yurovsky VV, **Gurbel PA**. Mild myocardial stunning affects platelet aggregation and certain hemostatic factors in swine. *Clin Appl Thromb Haemost* 5:236-42, 1999.
64. Murugesan SR, **Gurbel PA**, Serebruany VL. Storing paraformaldehyde fixed whole blood in patient samples after chronic platelet glycoprotein IIb/IIIa blockade: Core laboratory considerations. *Thromb Res* 95:201-203, 1999.
65. Serebruany VL, Kereiakes DJ, Dalesandro MR, **Gurbel PA**. The flow cytometer model markedly affects measurement of ex vivo whole blood platelet-bound P-selectin expression in patients with chest pain: Are we comparing apples and oranges? *Thromb Res* 96:51-56, 1999.
66. Serebruany VL, Yurovsky VV, **Gurbel PA**. Effects of a novel Mac-1 inhibitor, NPC 15669 on hemostatic parameters during preconditioned myocardial infarction. *Life Sciences* 65:1503-13, 1999.
67. **Gurbel PA**, Murugesan SR, Lowry DR, Serebruany VL. Plasma thromboxane and prostacyclin are linearly related and increased in patients presenting with myocardial infarction *Prostaglandins Leukot Essent Fatty Acids* 61:7-11,

1999.

68. McKenzie ME, **Gurbel PA**, Levine DJ, Serebruany VL. Clinical utility of available methods determining platelet function. *Cardiology* 92:240-7, 1999.
69. **Gurbel PA**, Kerelakes DJ, Dalesandro MR, Bahr RD, O'Connor CM, Serebruany VL. The role of soluble and platelet-bound P-selectin in discriminating cardiac from non-cardiac chest pain at presentation in the Emergency Department. *Am Heart J*. 139:320-328, 2000.
70. McKenzie ME, Pothula A, **Gurbel PA**, Fuzaylov SY, O'Connor CM, Gaitis WA, Serebruany VL. Failure of thrombin generation markers to triage patients presenting with chest pain. *Cardiology* 92:53-58, 2000.
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72. Pothula A, **Gurbel PA**, Atar D. McKenzie ME, Serebruany VL. Pathophysiology and therapeutic modification of thrombin generation in patients with coronary artery disease. *Eur J Pharmacol* 402:1-10, 2000.
73. Serebruany VL, Murugesan SR, Pothula, Atar D, Lowry DR, O'Connor CM, **Gurbel PA**. Increased soluble platelet/endothelial cellular adhesion molecule-1 and osteonectin levels in patients with severe congestive heart failure. Independence of disease etiology and antecedent aspirin therapy. *Eur J Heart Fail* 1:243-9, 2000.
74. Nair GV, **Gurbel PA**. Fusaylov SY, Davis CJ, Ohman EM, Bahr RD, Christensen RH, Serebruany VL. Combining necrosis and platelet markers for perfecting myocardial infarction rule out: How close are we? *Cardiology* 93:50-5, 2000.
75. Semann, HB, **Gurbel PA**, Anderson JL, Muhlestein JB, Carlquist JV, Home BD. Serebruany VL. Soluble VCAM-1 and E-selectin but not ICAM-1 discriminate endothelial injury in patients with documented coronary artery disease. *Cardiology* 93:7-10, 2000.
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Exhibit 8

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ACC/AHA Guidelines for the Management of Patients With Unstable Angina and Non-ST-Segment Elevation Myocardial Infarction: Executive Summary and Recommendations : A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on the Management of Patients With Unstable Angina)

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ACC/AHA Guidelines for the Management of Patients With Unstable Angina and Non-ST-Segment Elevation Myocardial Infarction: Executive Summary and Recommendations

A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on the Management of Patients With Unstable Angina)

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I. Introduction

The American College of Cardiology (ACC)/American Heart Association (AHA) Task Force on Practice Guidelines was formed to make recommendations regarding the diagnosis and treatment of patients with known or suspected cardiovascular disease. Coronary artery disease (CAD) is the leading cause of death in the United States. Unstable angina (UA) and the closely related condition non-ST-segment elevation myocardial infarction (NSTEMI) are very common manifestations of this disease. These life-threatening disorders are a major cause of emergency medical care and hospitalizations

in the United States. In 1996, the National Center for Health Statistics reported 1 433 000 hospitalizations for UA or NSTEMI. In recognition of the importance of the management of this common entity and of the rapid advances in the management of this condition, the need to revise guidelines published by the Agency for Health Care Policy and Research (AHCPR) and the National Heart, Lung and Blood Institute in 1994 was evident. This Task Force therefore formed the current committee to develop guidelines for the management of UA and NSTEMI. The present guidelines supersede the 1994 guidelines.

"ACC/AHA Guidelines for the Management of Patients With Unstable Angina and Non-ST-Segment Elevation Myocardial Infarction: Executive Summary and Recommendations: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Management of Patients With Unstable Angina)" was approved by the American College of Cardiology Board of Trustees in June 2000 and by the American Heart Association Science Advisory and Coordinating Committee in June 2000.

When citing this document, the American College of Cardiology and the American Heart Association request that the following citation format be used: Braunwald E, Antman EM, Beasley JW, Califf RM, Cheitlin MD, Hochman JS, Jones RH, Kereiakes D, Kupersmith J, Levin TN, Pepine CJ, Schaeffer JW, Smith EE III, Steward DE, Theroux P. ACC/AHA guidelines for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction: executive summary and recommendations: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Management of Patients With Unstable Angina). *Circulation* 2000;102:1193-1209. This document is available on the World Wide Web sites of the American College of Cardiology (www.acc.org) and the American Heart Association (www.americanheart.org). A single reprint of the executive summary and recommendations is available by calling 800-242-8721 (US only) or writing the American Heart Association, Public Information, 7272 Greenville Avenue, Dallas, TX 75231-4596. Ask for reprint No. 71-0187. To obtain a reprint of the complete guidelines published in the September issue of the *Journal of the American College of Cardiology*, ask for reprint No. 71-0188. To purchase additional reprints (specify version and reprint number): up to 999 copies, call 800-611-6083 (US only) or fax 413-665-2671; 1000 or more copies, call 214-706-1466, fax 214-691-6342, or e-mail pubauth@heart.org. To make photocopies for personal or educational use, call the Copyright Clearance Center, 978-750-8400.

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The customary ACC/AHA classifications I, II, and III summarize both the evidence and expert opinion and provide final recommendations for both patient evaluation and therapy:

Class I: Conditions for which there is evidence and/or general agreement that a given procedure or treatment is useful and effective.

Class II: Conditions for which there is conflicting evidence and/or a divergence of opinion about the usefulness/efficacy of a procedure or treatment.

Class IIa: Weight of evidence/opinion is in favor of usefulness/efficacy.

Class IIb: Usefulness/efficacy is less well established by evidence/opinion.

Class III: Conditions for which there is evidence and/or general agreement that the procedure/treatment is not useful/effective and in some cases may be harmful.

The weight of the evidence was ranked highest (A) if the data were derived from multiple randomized clinical trials that involved large numbers of patients and intermediate (B) if the data were derived from a limited number of randomized trials that involved small numbers of patients or from careful analyses of nonrandomized studies or observational registries. A low rank (C) was given when expert consensus was the primary basis for the recommendation.

The full text of the guidelines is published in the September 2000 issue of the *Journal of the American College of Cardiology*. This document was approved for publication by the governing bodies of the American College of Cardiology and the American Heart Association.

UA and NSTEMI are acute coronary syndromes (ACSs) that are characterized by an imbalance between myocardial oxygen supply and demand. The most common cause is reduced myocardial perfusion that results from coronary artery narrowing caused by a nonocclusive thrombus that has developed on a disrupted atherosclerotic plaque. Abnormal constriction of the coronary arteries may also be responsible. In the guidelines, UA and NSTEMI are considered to be closely related conditions whose pathogenesis and clinical presentations are similar but of differing severity (ie, they differ primarily in whether the ischemia is severe enough to cause sufficient myocardial damage to release detectable quantities of a marker of myocardial injury, most commonly, troponin I [TnI], troponin T [TnT], or the MB isoenzyme of creatine phosphokinase [CK-MB]). Once it has been established that no biochemical marker of myocardial necrosis has been released, the patient with an ACS may be considered to have experienced UA, whereas the diagnosis of NSTEMI is established if a marker of myocardial injury has been released.

II. Initial Evaluation and Management

A. Clinical Assessment

Recommendations for Initial Triage

Class I

1. Patients with symptoms that suggest possible ACS should not be evaluated solely over the telephone but

should be referred to a facility that allows evaluation by a physician and the recording of a 12-lead electrocardiogram (ECG). (Level of Evidence: C)

2. Patients with a suspected ACS with chest discomfort at rest for >20 minutes, hemodynamic instability, or recent syncope or presyncope should be strongly considered for immediate referral to an emergency department (ED) or a specialized chest pain unit. Other patients with a suspected ACS may be seen initially in an ED, a chest pain unit, or an outpatient facility. (Level of Evidence: C)

When symptoms have been unremitting for >20 minutes, the possibility of ST-segment elevation myocardial infarction (STEMI) must be considered. Given the strong evidence for a relationship between a delay in treatment and death for patients with STEMI, an immediate assessment that includes a 12-lead ECG is essential. Patients who are diagnosed as having an acute myocardial infarction (AMI) that is suitable for reperfusion should be managed as indicated according to the ACC/AHA Guidelines for the Management of Patients With Acute Myocardial Infarction.

B. Early Risk Stratification

Recommendations

Class I

1. A determination of the likelihood (high, intermediate, or low) of acute ischemia caused by CAD should be made in all patients with chest discomfort. (Level of Evidence: C)
2. Patients who present with chest discomfort should undergo early risk stratification that focuses on anginal symptoms, physical findings, ECG findings, and biomarkers of cardiac injury. (Level of Evidence: B)
3. A 12-lead ECG should be obtained immediately (within 10 minutes) in patients with ongoing chest discomfort and as rapidly as possible in patients who have a history of chest discomfort consistent with ACS but whose discomfort has resolved by the time of evaluation. (Level of Evidence: C)
4. Biomarkers of cardiac injury should be measured in all patients who present with chest discomfort consistent with ACS. A cardiac-specific troponin is the preferred marker, and if available, it should be measured in all patients. CK-MB by mass assay is also acceptable. In patients with negative cardiac markers within 6 hours of the onset of pain, another sample should be drawn in the 6- to 12-hour time frame (eg, at 9 hours after the onset of symptoms). (Level of Evidence: C)

Class IIa

1. For patients who present within 6 hours of the onset of symptoms, an early marker of cardiac injury (eg, myoglobin or CK-MB subforms) should be considered in addition to a cardiac troponin. (Level of Evidence: C)

Class IIb

1. C-reactive protein (CRP) and other markers of inflammation should be measured. (Level of Evidence: B)

TABLE 1. Short-Term Risk of Death or Nonfatal MI in Patients With UA

Feature	High Risk (At least 1 of the following features must be present)	Intermediate Risk (No high-risk feature but must have 1 of the following features)	Low Risk (No high- or intermediate-risk feature but may have any of the following features)
History	Accelerating tempo of ischemic symptoms in preceding 48 hrs	Prior MI, peripheral or cerebrovascular disease, or CABG; prior aspirin use	
Character of pain	Prolonged ongoing (>20 min) rest pain	Prolonged (>20 min) rest angina, now resolved, with moderate or high likelihood of CAD Rest angina (<20 min or relieved with rest or sublingual NTG)	New-onset CCS Class III or IV angina in the past 2 wk with moderate or high likelihood of CAD
Clinical findings	Pulmonary edema, most likely related to ischemia New or worsening MR murmur S ₃ or new/worsening rales Hypotension, bradycardia, tachycardia Age >75 y	Age >70 y	
ECG findings	Angina at rest with transient ST-segment changes >0.05 mV Bundle-branch block, new or presumed new Sustained ventricular tachycardia	T-wave inversions >0.2 mV Pathological Q waves	Normal or unchanged ECG during an episode of chest discomfort
Cardiac markers	Markedly elevated (eg, TnT or TnI >0.1 ng/mL)	Slightly elevated (eg, TnT >0.01 but <0.1 ng/mL)	Normal

An estimation of the short-term risks of death and nonfatal cardiac ischemic events in UA is a complex multivariable problem that cannot be fully specified in a table such as this. Therefore, the table is meant to offer general guidance and illustration rather than rigid algorithms.

Adapted with permission from Braunwald E, Mark DB, Jones RH, et al. Unstable angina: diagnosis and management. Rockville, MD: Agency for Health Care Policy and Research and the National Heart, Lung, and Blood Institute, US Public Health Service, US Department of Health and Human Services; 1994; AHCPR Publication No. 94-0602. AHCPR Clinical Practice Guideline No. 10, Unstable Angina: Diagnosis and Management, May 1994.

Class III

1. Total CK (without MB), aspartate aminotransferase (AST, SGOT), β -hydroxybutyric dehydrogenase, and/or lactate dehydrogenase should be the marker for the detection of myocardial injury in patients with chest discomfort suggestive of ACS. (Level of Evidence: C)

Estimation of the Level of Risk

The medical history, physical examination, ECG, and biochemical cardiac marker measurements in patients with symptoms suggestive of ACS at the time of initial presentation can be integrated into an estimate of the risk of death and nonfatal cardiac ischemic events. An estimation of the level of risk is a multivariable problem that cannot be simply quantified. Table 1 is illustrative of the general relationships between clinical and ECG findings and the categorization of patients into those at a low, an intermediate, or a high level of risk of events.

Because patients with new or severe ischemic discomfort are at an increased risk of cardiac death and nonfatal ischemic events, an assessment of the prognosis should set the pace of initial evaluation and treatment. An estimation of risk is useful in (1) selection of the site of care (coronary care unit, monitored step-down unit, or outpatient setting) and (2) selection of therapy, especially platelet glycoprotein (GP) IIb/IIIa inhibitors (see Section III. B) and coronary revascularization (see Section IV). For all modes of presentation of

an ACS, a strong relationship exists between indicators of the likelihood of ischemia due to CAD and prognosis. Therefore, an assessment of the likelihood of CAD is the starting point for determination of the prognosis of patients who present with symptoms that are suggestive of an ACS. Other important elements for prognostic assessment are the tempo of the patient's clinical course, which relates to the short-term risk of future cardiac events, principally AMI, and the patient's likelihood of survival should an acute ischemic event occur.

The 5 most important factors from the initial history that relate to the likelihood that the patient is experiencing an episode of ischemia due to CAD are (1) the nature of the symptoms, (2) a prior history of CAD, (3) age, (4) sex, and (5) the number of traditional risk factors that are present for CAD. Patients with UA may have discomfort that has all of the qualities of typical angina except that the episodes are more severe and prolonged, may occur at rest, or may be precipitated by less exertion than was previously necessary.

Recommendation for the Diagnosis of Noncardiac Causes of Symptoms

Class I

1. The initial evaluation of the patient with suspected ACS should include a search for noncoronary causes that could explain the development of symptoms. (Level of Evidence: C)

Information from the initial history, physical examination, and ECG will enable the physician to recognize and exclude from further assessment patients classified as "not having ischemic discomfort." This includes patients with noncardiac pain and patients with cardiac pain that is not caused by myocardial ischemia. The remaining patients should undergo a more complete evaluation of secondary causes of UA that might alter management. In patients with secondary angina, factors that increase myocardial oxygen demand or decrease oxygen delivery to the heart may provoke or exacerbate ischemia in the presence of significant underlying CAD.

The major objectives of the physical examination are to identify potential precipitating causes of myocardial ischemia (eg, uncontrolled hypertension or thyrotoxicosis) and evidence of other cardiac disease (eg, aortic stenosis or hypertrophic cardiomyopathy), and comorbid conditions (eg, pulmonary disease) and to assess the hemodynamic impact of the ischemic event.

Assessment of Risk of Death in Patients With UA/NSTEMI

The AHCPR guidelines "Unstable Angina: Diagnosis and Management" identified low-risk UA patients as those *without* rest or nocturnal angina and with normal or unchanged ECGs. High-risk patients were identified as those with pulmonary edema; ongoing rest pain for >20 minutes; angina with S₃ gallop, rales, or new or worsening mitral regurgitation murmur; hypotension; or dynamic ST-segment change of ≥ 1 mm. Patients without low- or high-risk features were termed to be at intermediate risk. The present guidelines endorse these principles (Table 1) but indicate that a rapid tempo of angina, a prior MI, and elevation of the cardiac-specific troponin level are also strong predictors of the risk of an adverse outcome. The *tempo of angina* is characterized by an assessment of changes in the duration of episodes, their frequency, and the anginal threshold.

Tools for Risk Stratification

Although imperfect, the 12-lead ECG lies at the center of the decision pathway for the evaluation and management of patients with ischemic discomfort. A recording made during an episode of the presenting symptoms is particularly valuable. Importantly, transient ST-segment changes (≥ 0.05 mV) that develop during a symptomatic episode at rest and that resolve when the patient becomes asymptomatic strongly suggest acute ischemia and a very high likelihood of underlying severe CAD.

CK-MB has until recently been the principal serum cardiac marker used in the evaluation of ACS. Despite its common use, CK-MB has several limitations (Table 2). Low levels of CK-MB in the blood of healthy individuals limit its specificity for myocardial necrosis. CK-MB levels may also be elevated with severe damage of skeletal muscle.

Monoclonal antibody-based immunoassays have been developed to detect cardiac-specific TnT (cTnT) and cardiac-specific TnI (cTnI). Because cTnT and cTnI are not detected in the blood of healthy individuals, the cutoff value for elevated cTnT and cTnI levels may be set to slightly above the upper limit of the assay of a normal healthy population,

leading to the terms "minor myocardial damage" or "micro-infarction" for patients with detectable troponin but no CK-MB in the blood. It is estimated that $\approx 30\%$ of patients who present with rest pain without ST-segment elevation and would otherwise be diagnosed as having UA because of a lack of CK-MB elevation actually have NSTEMI when assessed with cardiac-specific troponin assays.

Elevated levels of cTnT or cTnI convey prognostic information beyond that supplied by the clinical characteristics of the patient, the ECG at presentation, and a predischARGE exercise test. Furthermore, among patients without ST-segment elevation and normal CK-MB levels, elevated cTnI or cTnT concentrations identify those at an increased risk of death. Finally, there is a quantitative relationship between the quantity of cTnI or cTnT that is measured and the risk of death in patients who present with UA/NSTEMI (Figure 1). Patients who present without ST-segment elevation and have elevated cardiac-specific troponin levels may receive a greater treatment benefit from platelet GP IIb/IIIa inhibitors and low-molecular-weight heparin (LMWH).

Table 2 provides a comparison of the advantages and disadvantages of various cardiac markers for the evaluation and management of patients with suspected ACS but without ECG ST-segment elevation. The troponins offer greater diagnostic sensitivity due to their ability to identify patients with lesser amounts of myocardial damage. Nevertheless, these lesser amounts of damage are associated with a high risk in patients with ACSs, because they are thought to represent microinfarctions that result from microemboli from an unstable plaque. Cardiac-specific troponins are gaining acceptance as the primary biochemical cardiac marker in ACS. Although not quite as sensitive or specific as the troponins, CK-MB by mass assay remains a very useful marker for the detection of more than minor myocardial damage. A normal CK-MB level, however, does not exclude the minor myocardial damage and its attendant risk of adverse outcomes detectable with cardiac-specific troponins. Because of its poor cardiac specificity in the setting of skeletal muscle injury and its rapid clearance from the bloodstream, myoglobin should not be used as the *only* diagnostic marker for the identification of patients with NSTEMI, but its early appearance with myocardial injury makes its absence quite useful in ruling out myocardial necrosis.

When a central laboratory is used to measure biochemical cardiac markers, results should be available within 60 minutes and preferably within 30 minutes. Point-of-care systems, if implemented at the bedside, have the advantage of reducing delays due to transportation and processing in a central laboratory and can eliminate delays due to the lack of availability of central laboratory assays at all hours. These advantages of point-of-care systems must be weighed against the need for stringent quality control and appropriate training of ED personnel in assay performance.

Given the increasing interest in the hypothesis that destabilization of atherosclerotic plaques may result from inflammatory processes, several groups have evaluated markers of the acute phase of inflammation such as CRP in patients with UA. Patients who do not have biochemical evidence of

TABLE 2. Biochemical Cardiac Markers for the Evaluation and Management of Patients Suspected of Having an ACS but Without ST-Segment Elevation on 12-Lead ECG

Marker	Advantages	Disadvantages	Point of Care Test Available	Comment	Clinical Recommendation
CK-MB	1. Rapid, cost-efficient, accurate assays 2. Ability to detect early reinfarction	1. Loss of specificity in setting of skeletal muscle disease or injury including surgery 2. Low sensitivity during very early MI (<6 h after symptom onset) or later after symptom onset (>36 h) and for minor myocardial damage (detectable by troponins)	Yes	Familiar to majority of clinicians	Prior standard and still acceptable diagnostic test in most clinical circumstances
CK-MB Isoforms	Early detection of MI	1. Specificity profile similar to CK-MB 2. Current assays require special expertise	No	Experience to date predominantly in dedicated research centers	Useful for extremely early (3–6 h after symptom onset) detection of MI in centers with demonstrated familiarity with assay technique
Myoglobin	1. High sensitivity 2. Useful in early detection of MI 3. Detection of reperfusion 4. Most useful in ruling out MI	1. Very low specificity in setting of skeletal muscle injury or disease 2. Rapid return to normal range limits sensitivity for later presentations	Yes	More convenient early marker than CK-MB isoforms because of greater availability of assays for myoglobin Rapid-release kinetics make myoglobin useful for noninvasive monitoring of reperfusion in patients with established MI	Should not be used as only diagnostic marker because of lack of cardiac specificity
Cardiac troponins	1. Powerful tool for risk stratification 2. Greater sensitivity and specificity than CK-MB 3. Detection of recent MI up to 2 wk after onset 4. Useful for selection of therapy 5. Detection of reperfusion	1. Low sensitivity in very early phase of MI (<6 h after symptom onset) and requires repeat measurement at 8–12 h, if negative 2. Limited ability to detect late minor reinfarction	Yes	Data on diagnostic performance and potential therapeutic implications increasingly available from clinical trials	Useful as a single test to efficiently diagnose NSTEMI (including minor myocardial damage), with serial measurements; clinicians should familiarize themselves with diagnostic "cutoffs" used in their local hospital laboratory

myocardial necrosis but have an elevated CRP level appear to be at increased risk of an adverse outcome, especially those whose CRP levels are markedly elevated.

C. Immediate Management

Recommendations

Class I

1. The history, physical examination, 12-lead ECG, and initial cardiac marker tests should be integrated to assign patients with chest pain to 1 of 4 categories: a noncardiac diagnosis, chronic stable angina, possible ACS, and definite ACS. (Level of Evidence: C)
2. Patients with definite or possible ACS but whose initial 12-lead ECG and cardiac marker levels are normal should be observed in a facility with cardiac monitoring (eg, chest pain unit), and a repeat ECG and cardiac marker measurement should be obtained 6 to 12 hours after the onset of symptoms. (Level of Evidence: B)
3. If the follow-up 12-lead ECG and cardiac marker measurements are normal, a stress test (exercise or pharmacological) to provoke ischemia may be performed in the ED, in a chest pain unit, or on an outpatient basis shortly after discharge. Low-risk patients with a negative stress test can be managed as outpatients. (Level of Evidence: C)
4. Patients with definite ACS and ongoing pain, positive cardiac markers, new ST-segment deviations, new deep T-wave inversions, hemodynamic abnormalities, or a positive stress test should be admitted to the hospital for further management. (Level of Evidence: C)
5. Patients with possible ACS and negative cardiac markers who are unable to exercise or who have an abnormal resting ECG should have a pharmacological stress test. (Level of Evidence: B)
6. Patients with definite ACS and ST-segment elevation should be evaluated for immediate reperfusion therapy. (Level of Evidence: A)

Troponin I Levels to Predict the Risk of Mortality in Acute Coronary Syndromes

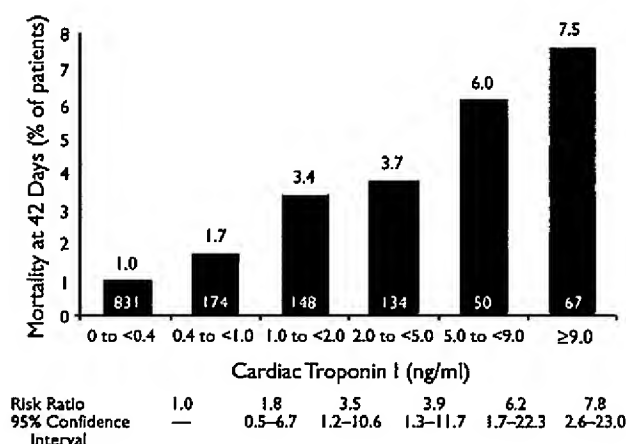


Figure 1. Relationship between cardiac troponin levels and risk of death in patients with ACS. Used with permission from Antman EM, Tanasijevic MJ, Thompson B, et al. Cardiac-specific troponin I levels to predict the risk of mortality in patients with acute coronary syndromes. *N Engl J Med*. 1996;335:1342–1349.

Through the integration of information from the history, physical examination, 12-lead ECG, and initial biochemical cardiac marker tests, clinicians can assign patients to 1 of 4 categories: noncardiac diagnosis, chronic stable angina, possible ACS, and definite ACS (Figure 2). Patients with

possible ACS are those who had a recent episode of chest discomfort at rest that was not entirely typical of ischemia but are pain free when initially evaluated, have a normal or unchanged ECG, and have no elevations of cardiac markers. Patients with a recent episode of typical ischemic discomfort that is either of new onset or severe or exhibits an accelerating pattern of previous stable angina (especially if it has occurred at rest or is within 2 weeks of a previously documented MI) should initially be considered to have *definite ACS*. However, such patients may be at low risk if the ECG obtained at presentation has no diagnostic abnormalities and the initial cardiac markers (especially a cardiac-specific troponin) are normal.

To facilitate a more definitive evaluation while avoiding the unnecessary hospital admission of patients with possible ACS and low-risk ACS and the inappropriate discharge of patients with active myocardial ischemia without ST elevation, special units have been devised that are variously referred to as “chest pain units” and “short-stay ED coronary care units.” These units use critical pathways or protocols designed to arrive at a decision about the presence or absence of myocardial ischemia and, if present, to characterize it as UA or NSTEMI and to define the optimal next step in the care of the patient (eg, discharge, admission, acute intervention). The goal is to arrive at such a decision after a finite amount of time, usually between 6 and 12 hours.

Patients who arrive at a medical facility in a pain-free state, have unchanged or normal ECGs, are hemodynamically

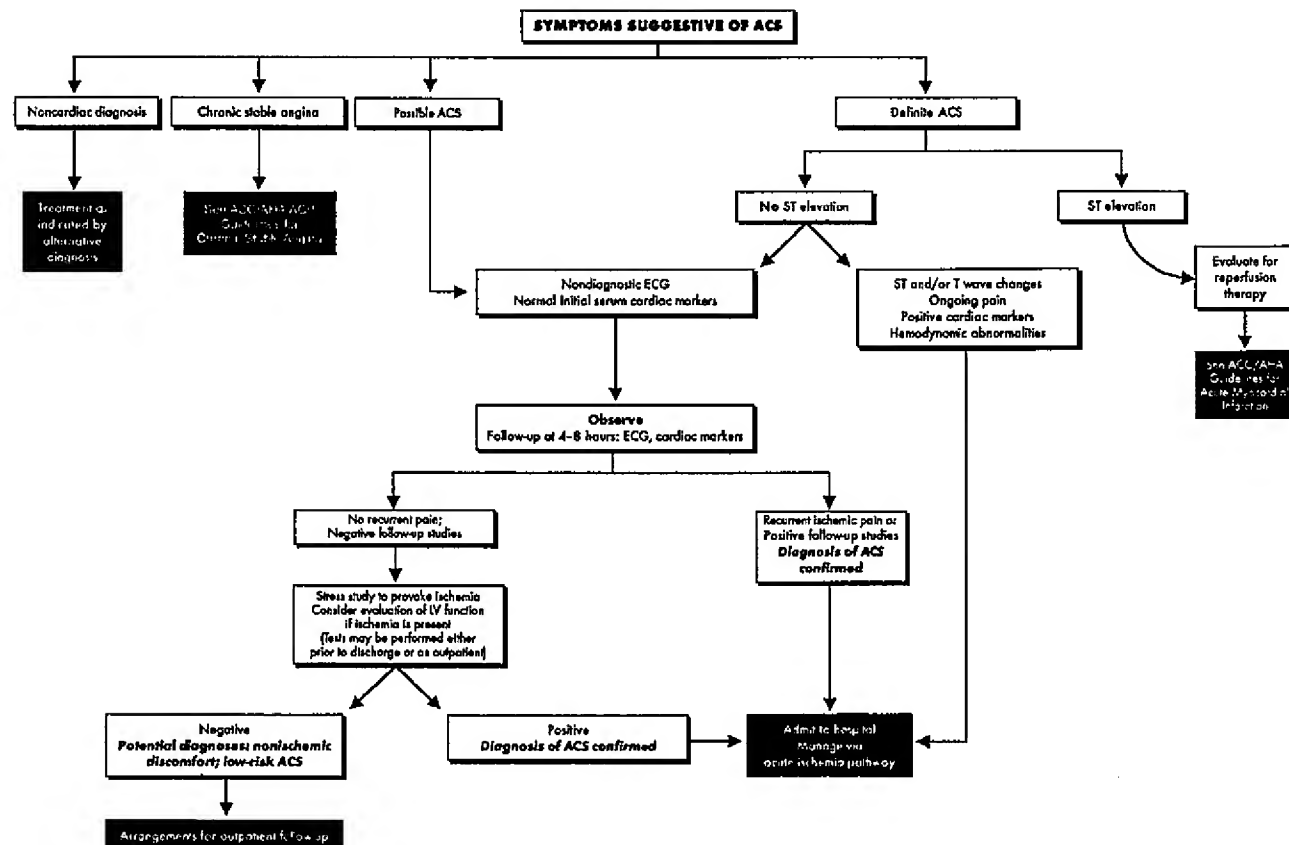


Figure 2. Algorithm for the evaluation and management of patients suspected of having an ACS.

stable, and do not have elevated cardiac markers represent more of a diagnostic than an urgent therapeutic challenge. Evaluation begins in these patients by obtaining information from the history, physical examination, and ECG (Table 1) to be used to confirm or reject the diagnosis of UA/NSTEMI. Patients with possible ACS are candidates for additional observation in a specialized facility (eg, chest pain unit). Patients with definite ACS are triaged based on the pattern of the 12-lead ECG. Patients with ST-segment elevation are evaluated for immediate reperfusion therapy and managed according to the ACC/AHA Guidelines for Management of Patients with Acute Myocardial Infarction, whereas those without ST-segment elevation are managed with either admission to the hospital or additional observation. During such observation, patients who experience recurrent ischemic discomfort, evolve abnormalities on a follow-up 12-lead ECG or cardiac marker measurement, or develop hemodynamic abnormalities such as new or worsening congestive heart failure (CHF) should be admitted to the hospital and managed as described in Section III. If the patient is at low risk (Table 1) and does not experience any further ischemic discomfort and his or her follow-up 12-lead ECG and cardiac marker measurements after 6 to 8 hours of observation remain normal, the patient may be considered for an early stress test to provoke ischemia. Patients discharged from the chest pain unit or ED should be counseled to make an appointment with their primary care physician as outpatients for further investigation into the cause of their symptoms. They should be seen by a physician within 72 hours of discharge from the ED or chest pain unit.

III. Hospital Care

The hospital care of patients with UA/NSTEMI is outlined in Figure 3.

A. Anti-Ischemic Therapy

Recommendations

Class I

1. Bed rest with continuous ECG monitoring for ischemia and arrhythmia detection in patients with ongoing rest pain. (Level of Evidence: C)
2. Nitroglycerin (NTG), sublingual tablet or spray, followed by intravenous administration, for immediate relief of ischemia and associated symptoms. (Level of Evidence: C)
3. Supplemental oxygen for patients with cyanosis or respiratory distress; finger pulse oximetry or arterial blood gas determination to confirm adequate arterial oxygen saturation ($\text{Sao}_2 > 90\%$) and continued need for supplemental oxygen in the presence of hypoxemia. (Level of Evidence: C)
4. Morphine sulfate intravenously when symptoms are not immediately relieved with NTG or when acute pulmonary congestion and/or severe agitation is present. (Level of Evidence: C)
5. A β -blocker, with the first dose administered intravenously if there is ongoing chest pain, followed by oral administration, in the absence of contraindications. (Level of Evidence: B)

6. In patients with continuing or frequently recurring ischemia when β -blockers are contraindicated, a nondihydropyridine calcium antagonist (eg, verapamil or diltiazem), followed by oral therapy, as initial therapy in the absence of severe LV dysfunction or other contraindications. (Level of Evidence: B)
7. An ACEI when hypertension persists despite treatment with NTG and a β -blocker in patients with LV systolic dysfunction or CHF and in ACS patients with diabetes. (Level of Evidence: B)

Class IIa

1. Oral long-acting calcium antagonists for recurrent ischemia in the absence of contraindications and when β -blockers and nitrates are fully used. (Level of Evidence: C)
2. An ACEI for all post-ACS patients. (Level of Evidence: B)
3. Intra-aortic balloon pump counterpulsation for severe ischemia that is continuing or recurs frequently despite intensive medical therapy or for hemodynamic instability in patients before or after coronary angiography. (Level of Evidence: C)

Class IIb

1. Extended-release form of nondihydropyridine calcium antagonists instead of a β -blocker. (Level of Evidence: B)
2. Immediate-release dihydropyridine calcium antagonists in the presence of a β -blocker. (Level of Evidence: B)

Class III

1. NTG or other nitrate within 24 hours of sildenafil (Viagra) use. (Level of Evidence: C)
2. Immediate-release dihydropyridine calcium antagonists in the absence of a β -blocker. (Level of Evidence: A)

Patients should be placed at bed rest while ischemia is ongoing but can be mobilized to a chair and bedside commode when symptom free. Patients with cyanosis, respiratory distress, or other high-risk features should receive supplemental oxygen. Adequate arterial oxygen saturation should be confirmed with direct measurement or pulse oximetry. Inhaled oxygen should be administered if the arterial oxygen saturation (Sao_2) declines to $<90\%$. Finger pulse oximetry is useful for continuous monitoring of Sao_2 but is not mandatory in patients who do not appear to be at risk of hypoxia. Patients should undergo continuous ECG monitoring during their ED evaluation and early hospital phase, because sudden, unexpected ventricular fibrillation is the major preventable cause of death in this early period. Furthermore, monitoring for recurrence of ST-segment shifts provides useful diagnostic and prognostic information, although the system of monitoring for ST-segment shifts must include specific methods intended to provide stable and accurate recordings.

Patients whose symptoms are not relieved with three 0.4-mg sublingual nitroglycerin (NTG) tablets or spray taken 5 minutes apart and initiation of an intravenous β -blocker (when there are no contraindications), as well as all nonhypotensive high-risk patients (Table 1), may benefit from

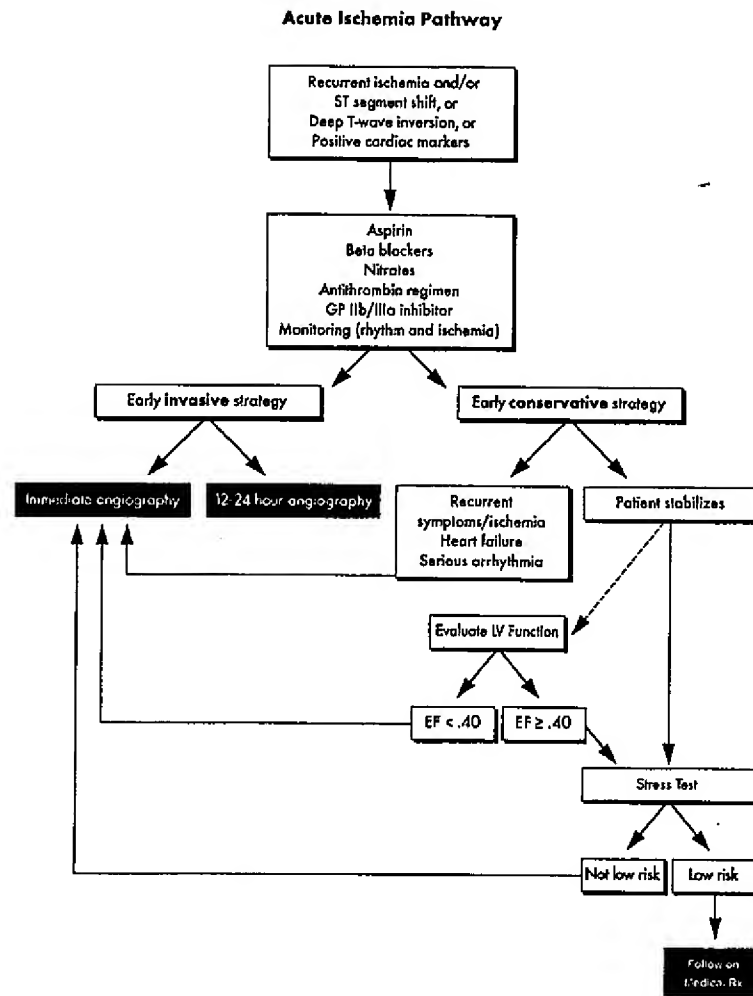


Figure 3. Acute ischemia pathway. Rx indicates therapy.

intravenous NTG, and such therapy is recommended in the absence of contraindications (ie, the use of sildenafil within the previous 24 hours or hypotension). Intravenous NTG may be initiated at a rate of 10 $\mu\text{g}/\text{min}$ via continuous infusion with nonabsorbing tubing and increased by 10 $\mu\text{g}/\text{min}$ every 3 to 5 minutes until some symptomatic or blood pressure response is noted.

Topical or oral nitrates are acceptable alternatives for patients without ongoing refractory symptoms. Tolerance to the hemodynamic effects of nitrates is dose and duration dependent and typically becomes important after 24 hours of continuous therapy with any formulation. Patients who require continued intravenous NTG beyond 24 hours may require periodic increases in the infusion rate to maintain efficacy. An effort must be made to use non-tolerance-producing nitrate regimens (lower dose and intermittent dosing).

Morphine sulfate at a rate of 1 to 5 mg IV is recommended for patients whose symptoms are not relieved after 3 serial sublingual NTG tablets or whose symptoms recur despite adequate anti-ischemic therapy. Unless contraindicated by hypotension or intolerance, morphine may be administered along with intravenous NTG, with careful blood pressure monitoring, and may be repeated every 5 to 30 minutes as needed to relieve symptoms and maintain patient comfort.

β -Blockers should be started early in the absence of contraindications. These agents should be administered intravenously, followed by oral administration, in high-risk patients, as well as in patients with ongoing rest pain, or orally for intermediate- and low-risk patients. Several regimens may be used. For example, intravenous metoprolol may be administered in 5-mg increments via slow intravenous administration (5 mg every 1 to 2 minutes) and repeated every 5 minutes for a total initial dose of 15 mg. In patients who tolerate the total 15-mg intravenous dose, oral therapy should be initiated 15 minutes after the last intravenous dose at 25 to 50 mg every 6 hours for 48 hours. Thereafter, patients should receive a maintenance dose of 100 mg twice daily. Monitoring during intravenous β -blocker therapy should include frequent checks of heart rate and blood pressure and continuous ECG monitoring, as well as auscultation for rales and bronchospasm.

Calcium antagonists may be used to control ongoing or recurring ischemia-related symptoms in patients who are already receiving adequate doses of nitrates and β -blockers, in patients who are unable to tolerate adequate doses of 1 or both of these agents, or in patients with variant angina (see Section VI. F). In addition, these drugs have been used for the management of hypertension in patients with recurrent UA. Rapid-release, short-acting dihydropyridines (eg, nifedipine)

must be avoided in the absence of adequate concurrent β -blockade in ACS, because controlled trials suggest increased adverse outcomes. When β -blockers cannot be used, heart rate–slowing calcium antagonists (eg, verapamil or diltiazem) offer an alternative. When required for the control of refractory symptoms, these agents can be used early during the hospital phase even in patients with mild left ventricular (LV) dysfunction, although the combination of a β -blocker and calcium antagonist may act in synergy to depress LV function.

Angiotensin-converting enzyme inhibitors (ACEIs) have been shown to reduce mortality rates in patients with AMI and in patients with recent MI or with LV systolic dysfunction, in diabetic patients with LV dysfunction, and in a broad spectrum of patients with high-risk chronic CAD. Accordingly, ACEIs should be used in such patients as well as in those with hypertension that is not controlled with β -blockers and nitrates.

B. Antiplatelet and Anticoagulation Therapy Recommendations

Class I

1. Antiplatelet therapy should be initiated promptly. Aspirin (ASA) is the first choice and is administered as soon as possible after presentation and continued indefinitely. (Level of Evidence: A)
2. A thienopyridine (clopidogrel or ticlopidine) should be administered to patients who are unable to take ASA because of hypersensitivity or major gastrointestinal intolerance. (Level of Evidence: B)
3. Parenteral anticoagulation with intravenous unfractionated heparin (UFH) or with subcutaneous LMWH should be added to antiplatelet therapy with ASA, or a thienopyridine. (Level of Evidence: B)
4. A platelet GP IIb/IIIa receptor antagonist should be administered, in addition to ASA and UFH, to patients with continuing ischemia or with other high-risk features (see Table 2) and to patients in whom a percutaneous coronary intervention (PCI) is planned. Eptifibatide and tirofiban are approved for this use. (Level of Evidence: A) Abciximab can also be used for 12 to 24 hours in patients with UA/NSTEMI in whom a PCI is planned within the next 24 hours. (Level of Evidence: A)

Class III

1. Intravenous thrombolytic therapy in patients without acute ST-segment elevation, a true posterior MI, or a presumed new left bundle-branch block. (Level of Evidence: A)

Antithrombotic therapy is essential to modify the disease process and its progression to death, MI, or recurrent MI. A combination of ASA, UFH, and a platelet GP IIb/IIIa receptor antagonist represents the most effective therapy. The intensity of treatment is tailored to individual risk, and triple antithrombotic treatment should be used in patients with continuing ischemia or with other high-risk features and in patients in whom an early invasive strategy is planned.

Some of the strongest evidence available about the long-term prognostic effects of therapy in CAD patients pertains to ASA. Among all clinical investigations with ASA, trials in UA/NSTEMI have most consistently documented a striking benefit of the drug despite differences in study design, such as time of entry after the acute phase, duration of follow-up, and doses. ASA should be initiated at a daily dose of 160 or 325 mg in patients with UA/NSTEMI. In patients who present with suspected ACS who are not already receiving ASA, the first dose may be chewed to establish a high blood level rapidly. Subsequent doses may be swallowed. Thereafter, daily doses of 75 to 325 mg are prescribed.

Few contraindications to ASA exist; these are intolerance and allergy (primarily manifested as asthma), active bleeding, hemophilia, active retinal bleeding, severe untreated hypertension, an active peptic ulcer, or another serious source of gastrointestinal or genitourinary bleeding. Gastrointestinal side effects such as dyspepsia and nausea are infrequent with the low doses.

Two thienopyridines, ticlopidine and clopidogrel, are adenosine diphosphate (ADP) antagonists that are currently approved for antiplatelet therapy. The platelet effects of ticlopidine and clopidogrel are irreversible but take several days to become completely manifest. The adverse effects of ticlopidine limit its usefulness and include gastrointestinal problems (eg, diarrhea, abdominal pain, nausea, vomiting), neutropenia in $\approx 2.4\%$ of patients, severe neutropenia in 0.8% of patients, and, rarely, thrombotic thrombocytopenia purpura (TTP). Neutropenia usually resolves within 1 to 3 weeks of the discontinuation of therapy but very rarely may be fatal.

Ticlopidine and clopidogrel are useful antiplatelet drugs for secondary prevention with an efficacy at least similar to that of ASA. These drugs are indicated in patients with UA/NSTEMI who are unable to tolerate ASA due to either hypersensitivity or major gastrointestinal contraindications—principally recent significant bleeding from a peptic ulcer or gastritis. Care must be taken during the acute phase with these drugs because of the delays required to achieve a full antiplatelet effect. Clopidogrel is preferred to ticlopidine because it has a more favorable safety profile.

Heparin is a key component in the antithrombotic management of UA/NSTEMI. The results of the studies that have compared the combination of ASA and either UFH or LMWH with the use of ASA alone have shown reductions in the rate of death or MI during the first week of 50% to 60%.

UFH has important pharmacokinetic limitations that are related to its nonspecific binding to proteins and cells. These limitations translate into poor bioavailability, especially at low doses, and marked variability in anticoagulant response among patients. As a consequence, the anticoagulant effect of UFH requires monitoring according to the activated partial thromboplastin time. The dose of UFH should be titrated to an activated partial thromboplastin time that is 1.5 to 2.5 times control. Serial hemoglobin/hematocrit and platelet measurements should be taken at least daily during UFH therapy. Advantages of LMWH preparations are the ease of subcutaneous administration and the absence of a need for monitoring. Furthermore, the LMWHs stimulate platelets less than does UFH and are less frequently associated with heparin-

induced thrombocytopenia. However, they appear to be associated with significantly more frequent *minor, but not major*, bleeding.

Two trials with enoxaparin, an LMWH, have shown a moderate benefit over UFH, and 2 trials, 1 with dalteparin and 1 with nadroparin, have shown neutral or unfavorable trends. A meta-analysis of the 2 trials with enoxaparin that involves a total of 7081 patients showed a statistically significant reduction of $\approx 20\%$ in the rate of death, MI, or urgent revascularization and in the rate of death or MI at 8, 14, and 43 days. There was a trend toward a reduction in death as well.

Platelet GP IIb/IIIa Receptor Antagonists

The GP IIb/IIIa receptor ($\alpha_{IIb}\beta_3$ integrin) is abundant on the platelet surface. When platelets are activated, this receptor undergoes a change in configuration that increases its affinity for binding to fibrinogen and other ligands. Binding of molecules of fibrinogen to receptors on different platelets results in platelet aggregation. This mechanism is independent of the stimulus for platelet aggregation and represents the final and obligatory pathway for platelet aggregation. The platelet GP IIb/IIIa receptor antagonists act by preventing fibrinogen binding and thereby preventing platelet aggregation.

The various GP IIb/IIIa antagonists, however, possess significantly different pharmacokinetic and pharmacodynamic properties. Abciximab is a Fab fragment of a humanized murine antibody that has a short plasma half-life but strong affinity for the receptor, resulting in some receptor occupancy that persists for weeks. Platelet aggregation gradually returns to normal 24 to 48 hours after the discontinuation of the drug. Abciximab is not specific for GP IIb/IIIa and inhibits the vitronectin receptor ($\alpha_v\beta_3$) on endothelial cells and the MAC-1 receptor on leukocytes as well. Eptifibatide is a cyclic heptapeptide that contains the KGD (Lys-Gly-Asp) sequence; tirofiban is a nonpeptide mimetic of the RGD (Arg-Gly-Asp) sequence of fibrinogen. Receptor occupancy with these 2 synthetic antagonists is in general in equilibrium with plasma levels. They have a half-life of 2 to 3 hours and are highly specific for the GP IIb/IIIa receptor, with no effect on the vitronectin receptor ($\alpha_v\beta_3$ integrin).

The efficacy of GP IIb/IIIa antagonists in prevention of the complications associated with percutaneous interventions has been documented in numerous trials, many of which are composed entirely or in large part of patients with UA. Two trials with tirofiban and 1 trial with eptifibatide have also documented their efficacy in UA/NSTEMI patients, of whom only some underwent interventions. Abciximab has been studied primarily in PCI trials, in which its administration consistently showed a significant reduction in the rate of MI and the need for urgent revascularization. Because the various agents have not been compared directly with each other, their *relative* efficacy is not known.

The cumulative event rates observed during the phase of medical management and at the time of PCI in the c7E3 Fab Antiplatelet Therapy in Unstable Refractory Angina (CAPTURE) (abciximab), Platelet Receptor Inhibition in Ischemic Syndrome Management in Patients Limited by

Unstable Signs and Symptoms (PRISM-PLUS) (tirofiban), and Platelet Glycoprotein IIb/IIIa in Unstable Angina: Receptor Suppression Using Integrilin Therapy (PURSUIT) (eptifibatide) trials are shown in Figure 4. Each trial has shown a statistically significant reduction in the rate of death or MI during the phase of medical management; the reduction in event rates was magnified at the time of the intervention.

Treatment with a GP IIb/IIIa antagonist increases the risk of bleeding, which is typically mucocutaneous or involves the access site of vascular intervention. No trials have shown an excess of intracranial bleeding with a GP IIb/IIIa inhibitor. Blood hemoglobin and platelet counts should be monitored and patient surveillance for bleeding should be carried out daily during the administration of GP IIb/IIIa receptor blockers. Thrombocytopenia is an unusual complication of this class of agents. ASA has been used with the intravenous GP IIb/IIIa receptor blockers in all trials. A strong case can also be made for the concomitant use of heparin with GP IIb/IIIa receptor blockers. Information is currently being gained concerning the safety and efficacy of the combination of LMWH and GP IIb/IIIa inhibitors.

The failure of intravenous thrombolytic therapy to improve clinical outcomes in UA/NSTEMI has been clearly demonstrated in several trials.

C. Risk Stratification

Recommendations

Class I

1. Noninvasive stress testing in low-risk patients (Table 1) who have been free of ischemia at rest or with low-level activity and of CHF for a minimum of 12 to 24 hours. (Level of Evidence: C)
2. Noninvasive stress testing in patients at intermediate risk (Table 1) who have been free of ischemia at rest or with low-level activity and of CHF for a minimum of 2 or 3 days. (Level of Evidence: C)
3. Choice of stress test is based on the resting ECG, ability to perform exercise, local expertise, and technologies available. Treadmill exercise is suitable in patients able to exercise in whom the ECG is free of baseline ST-segment abnormalities, bundle-branch block, LV hypertrophy, intraventricular conduction defect, paced rhythm, preexcitation, or digoxin effect. (Level of Evidence: C)
4. An imaging modality is added in patients with resting ST-segment depression (≥ 0.10 mV), LV hypertrophy, bundle-branch block, intraventricular conduction defect, preexcitation, or digoxin who are able to exercise. In patients undergoing a low-level exercise test, an imaging modality may add sensitivity. (Level of Evidence: C)
5. Pharmacological stress testing with imaging when physical limitations (eg, arthritis, amputation, severe peripheral vascular disease, severe chronic obstructive pulmonary disease, general debility) preclude adequate exercise stress. (Level of Evidence: B)
6. Prompt angiography without noninvasive risk stratification for failure of stabilization with intensive medical treatment. (Level of Evidence: B)

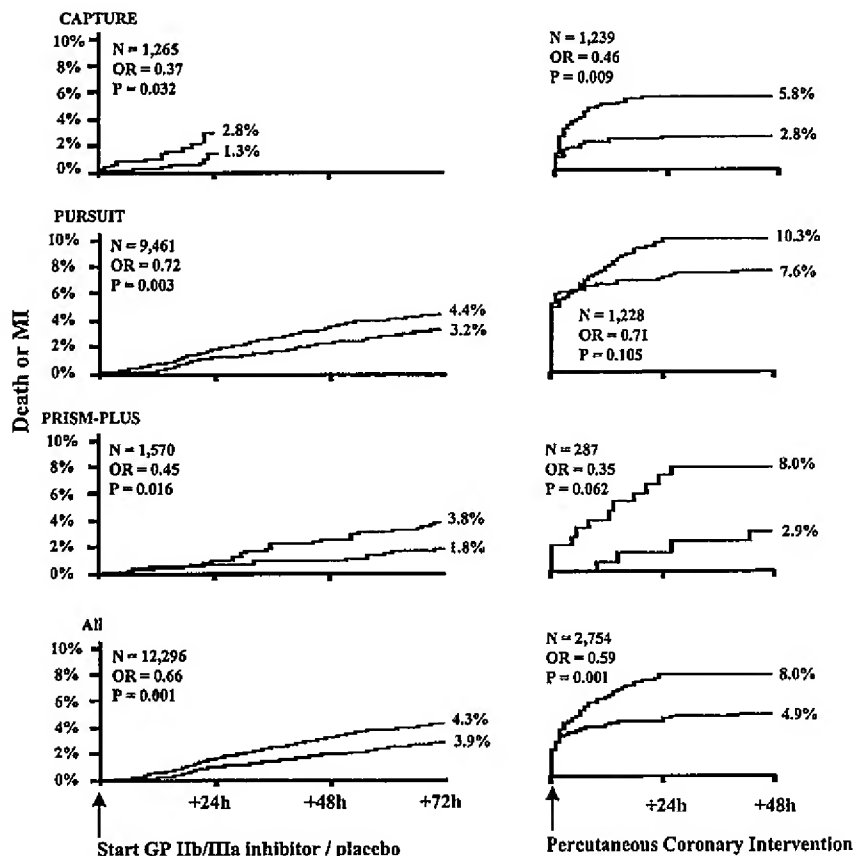


Figure 4. Kaplan-Meier curves showing cumulative incidence of death or MI in patients randomly assigned to platelet GP IIb/IIIa receptor antagonist (bold line) or placebo. Data are derived from the CAPTURE, PURSUIT, and PRISM-PLUS trials. Left, Events during the initial period of medical treatment until the moment of PCI or CABG. In the CAPTURE trial, abciximab was administered for 18 to 24 hours before the PCI was performed in almost all patients as per study design; abciximab was discontinued 1 hour after the intervention. In PURSUIT, a PCI was performed in 11.2% of patients during a period of medical therapy with eptifibatide that lasted 72 hours and for 24 hours after the intervention. In PRISM-PLUS, an intervention was performed in 30.2% of patients after a 48-hour period of medical therapy with tirofiban, and the drug infusion was maintained for 12 to 24 hours after an intervention. Right, Events occurring at the time of PCI and the next 48 hours, with the event rates reset to 0% before the intervention. CK or CK-MB elevations exceeding 2 times the upper limit of normal were considered as infarction during medical management and exceeding 3 times the upper limit of normal for PCI-related events. OR indicates odds ratio. Adapted with permission from Boersma E, Akkerhuis KM, Theroux P, et al. Platelet glycoprotein IIb/IIIa receptor inhibition in non-ST-elevation acute coronary syndromes: early benefit during medical treatment only, with additional protection during percutaneous coronary intervention. *Circulation*. 1999;100:2045-2048.

Class IIa

1. A noninvasive test (echocardiogram or radionuclide angiogram) to evaluate LV function in patients with definite ACS who are not scheduled for coronary arteriography and left ventriculography. (Level of Evidence: C)

The management of patients with an ACS requires continuous risk stratification. Important prognostic information is derived from a careful initial assessment and the patient's course over the first few days of management and the response to anti-ischemic and antithrombotic therapy. The goals of noninvasive testing are to (1) determine the presence or absence of ischemia in patients at low likelihood of CAD and (2) estimate prognosis.

Because of simplicity, lower cost, and widespread familiarity with performance and interpretation, the standard low-level exercise ECG stress test remains the most reasonable test in patients able to exercise who have a resting ECG that is interpretable for ST-segment shifts. Patients with an ECG pattern that would interfere with interpretation of the ST segment should have an exercise test with imaging. Patients who are unable to exercise should have a pharmacological stress test with imaging. A low-level exercise test (eg, to completion of Bruce Stage II) may be carried out in low-risk patients (Table 1) who have been asymptomatic for 12 to 24 hours. A symptom-limited test can be conducted in patients without evidence of ischemia for 7 to 10 days.

In contrast to the noninvasive tests, coronary angiography provides detailed structural information to allow an assess-

ment of the prognosis and to provide direction for appropriate management. When combined with LV angiography, it also allows an assessment of global and regional LV function. In patients with UA/NSTEMI, coronary angiography typically shows the following profile: (1) no severe epicardial stenosis in 10% to 20% of patients, (2) significant (>50%) left main stenosis in 5% to 10% of patients, (3) multivessel stenosis in 40% to 50% of patients, and (4) 1-vessel stenosis in 30% to 35% of patients.

D. Early Conservative Versus Invasive Strategies

Two different treatment strategies, termed "early conservative" and "early invasive," have evolved for patients with UA/NSTEMI. In the early conservative strategy, coronary angiography is reserved for patients with evidence of recurrent ischemia (angina or ST-segment changes at rest or with minimal activity) or a strongly positive stress test despite vigorous medical therapy. In the early invasive strategy, patients without clinically obvious contraindications to coronary revascularization are routinely recommended for coronary angiography and angiographically directed revascularization if possible.

Recommendations

Class I

1. An early invasive strategy in patients with UA/NSTEMI and any of the following high-risk indicators (Level of Evidence: B):

- a) Patients with recurrent angina/ischemia at rest or with low-level activities despite intensive anti-ischemic therapy
 - b) Recurrent angina/ischemia with CHF symptoms, an S₃ gallop, pulmonary edema, worsening rales, or new or worsening mitral regurgitation
 - c) High-risk findings on noninvasive stress testing
 - d) Depressed LV systolic function (eg, EF <0.40 on noninvasive study)
 - e) Hemodynamic instability or angina at rest accompanied by hypotension
 - f) Sustained ventricular tachycardia
 - g) PCI within 6 months
 - h) Prior CABG
2. In the absence of these findings, either an early conservative or an early invasive strategy in hospitalized patients without contraindications for revascularization. (Level of Evidence: B)

Class IIa

1. An early invasive strategy in patients with repeated presentations for ACS despite therapy and without evidence of ongoing ischemia or high risk. (Level of Evidence: C)
2. An early invasive strategy in patients >65 years old or patients who present with ST-segment depression or elevated cardiac markers and no contraindications to revascularization. (Level of Evidence: C)

Class III

1. Coronary angiography in patients with extensive comorbidities (eg, liver or pulmonary failure, cancer), in whom risks of revascularization are not likely to outweigh the benefits. (Level of Evidence: C)
2. Coronary angiography in patients with acute chest pain and a low likelihood of ACS. (Level of Evidence: C)
3. Coronary angiography in patients who will not consent to revascularization regardless of the findings. (Level of Evidence: C)

Rationale for the Early Invasive Strategy

In patients with UA/NSTEMI without recurrent ischemia in the first 24 hours, the use of early angiography provides a convenient approach to risk stratification. It can identify the patients with no significant coronary stenoses and those with 3-vessel disease with LV dysfunction or left main disease. The former group has an excellent prognosis, whereas the latter group may derive a survival benefit from coronary artery bypass graft surgery (CABG) (see Section IV). In addition, early percutaneous revascularization of the culprit lesion has the potential to reduce the risk for subsequent hospitalization and the need for multiple antianginal drugs compared with the early conservative strategy. Some believe that proceeding immediately to angiography is an efficient approach for the ACS patient. Others believe that 12 to 48 hours of anti-ischemic or antithrombotic therapy is preferable.

In a patient with UA, a history of *prior PCI* within the past 6 months suggests the presence of restenosis, which often can be effectively treated with repeat PCI. Coronary angiography without preceding functional testing is generally indicated.

Patients with *prior CABG* represent another subgroup for whom a strategy of early coronary angiography is generally indicated. In addition, patients with known or suspected *reduced LV systolic function*, including patients with prior anterior Q-wave MIs, those with prior measurements that show depressed LV function, or those who present with CHF, have sufficient risk that the possibility of benefit from revascularization procedures merits early coronary angiography without preceding functional testing.

Rationale for the Early Conservative Strategy

Clinical evaluation and noninvasive testing aid in the identification of most patients who require revascularization, because they have markers of high risk, such as advanced age (>70 years), prior MI, revascularization, ST-segment deviation, CHF, or depressed resting LV function (ie, EF <0.40) on noninvasive study or noninvasive stress test findings that suggest severe ischemia. The remaining larger subgroup of patients, however, do not have the findings that portend a high risk for adverse outcomes. Accordingly, they are not likely to receive such benefit from routine revascularization, and coronary arteriography is optional in them. It can be safely deferred pending further clinical developments. Decisions regarding coronary angiography in patients who are *not* high risk according to findings on clinical examination and noninvasive testing can be individualized based on patient preferences.

IV. Coronary Revascularization

Coronary revascularization (PCI or CABG) is carried out to improve prognosis, relieve symptoms, prevent ischemic complications, and improve functional capacity. The decision to proceed from diagnostic angiography to revascularization is influenced not only by the coronary anatomy but also by a number of additional factors, including anticipated life expectancy, ventricular function, comorbidity, functional capacity, severity of symptoms, and quantity of viable myocardium at risk. These are all important variables that must be considered before revascularization is recommended. For example, patients with distal obstructive coronary lesions or those who have large quantities of irreversibly damaged myocardium are unlikely to benefit from revascularization, particularly if they can be stabilized on medical therapy. Patients with high-risk coronary anatomy are likely to benefit from revascularization in terms of both symptom improvement and long-term survival. The indications for coronary revascularization in patients with UA/NSTEMI are similar to those for patients with chronic stable angina (see the ACC/AHA/ACP-ASIM Guidelines for the Management of Patients With Chronic Stable Angina and the ACC/AHA Guidelines for Coronary Artery Bypass Graft Surgery).

Recommendations for Revascularization With PCI and CABG in Patients With UA/NSTEMI

Class I

1. CABG for patients with significant left main CAD. (Level of Evidence: A)

2. CABG for patients with 3-vessel disease; the survival benefit is greater in patients with abnormal LV function (EF <0.50). (Level of Evidence: A)
3. CABG for patients with 2-vessel disease with significant proximal left anterior descending CAD and either abnormal LV function (EF <0.50) or demonstrable ischemia on noninvasive testing. (Level of Evidence: A)
4. PCI or CABG for patients with 1- or 2-vessel CAD without significant proximal left anterior descending CAD but with a large area of viable myocardium and high-risk criteria on noninvasive testing. (Level of Evidence: B)
5. PCI for patients with multivessel coronary disease with suitable coronary anatomy, with normal LV function, and without diabetes. (Level of Evidence: A)
6. Intravenous platelet GP IIb/IIIa inhibitor in UA/NSTEMI patients undergoing PCI. (Level of Evidence: A)

Class IIa

1. Repeat CABG for patients with multiple saphenous vein graft (SVG) stenoses, especially when there is significant stenosis of a graft that supplies the left anterior descending coronary artery (LAD). (Level of Evidence: C)
2. PCI for focal SVG lesions or multiple stenoses in poor candidates for reoperative surgery. (Level of Evidence: C)
3. PCI or CABG for patients with 1- or 2-vessel CAD without significant proximal left anterior descending CAD but with a moderate area of viable myocardium and ischemia on noninvasive testing. (Level of Evidence: B)
4. PCI or CABG for patients with 1-vessel disease with significant proximal left anterior descending CAD. (Level of Evidence: B)
5. CABG with the internal mammary artery for patients with multivessel disease and treated diabetes mellitus. (Level of Evidence: B)

Class IIb

1. PCI for patients with 2- or 3-vessel disease with significant proximal left anterior descending CAD, with treated diabetes or abnormal LV function, and with anatomy suitable for catheter-based therapy. (Level of Evidence: B)

Class III

1. PCI or CABG for patients with 1- or 2-vessel CAD without significant proximal left anterior descending CAD or with mild symptoms or symptoms that are unlikely to be due to myocardial ischemia or who have not received an adequate trial of medical therapy and who have no demonstrable ischemia on noninvasive testing. (Level of Evidence: C)
2. PCI or CABG for patients with insignificant coronary stenosis (<50% diameter). (Level of Evidence: C)
3. PCI in patients with significant left main coronary artery disease who are candidates for CABG. (Level of Evidence: B)

Percutaneous coronary revascularization (intervention) strategies are referred to in the guidelines as "PCI." The majority of current PCIs involve balloon dilatation and coronary stenting. Stenting has contributed greatly to catheter-based revascularization by reducing the risk of both acute vessel closure and late restenosis.

Platelet Inhibitors and Percutaneous Revascularization

Data from both retrospective observations and randomized clinical trials indicate that PCI can lead to angiographic success in most patients with UA/NSTEMI. An important advance in the treatment of patients with UA/NSTEMI undergoing PCI has been the introduction of platelet GP IIb/IIIa receptor inhibitors (see Section III). This therapy takes advantage of the fact that platelets play an important role in the development of ischemic complications that may occur in patients with UA/NSTEMI or during coronary revascularization procedures. The safety of these procedures in these patients is enhanced by the addition of intravenous platelet GP IIb/IIIa receptor inhibitors to the standard regimen of ASA, heparin, and anti-ischemic medications.

Percutaneous Transluminal Coronary Angioplasty Versus CABG

A meta-analysis of 8 randomized trials completed between 1986 and 1993 has been carried out that compared the outcomes of CABG and percutaneous transluminal coronary angioplasty (PTCA) in 3371 patients with multivessel CAD (many of whom presented with UA). At 1-year follow-up, no difference was documented between the 2 therapies in cardiac death or MI, but a lower incidence of angina and need for revascularization was associated with CABG. Subsequently, the results were reported of the Bypass Angioplasty Revascularization Investigation (BARI) trial, the largest randomized comparison of CABG and PTCA, which was conducted in 1829 patients with 2- or 3-vessel CAD; UA was the admitting diagnosis in 64% of these patients. A statistically significant advantage in survival without MI independent of the severity of presenting symptoms was observed in the entire group for CABG compared with PCI at 7 years after study entry (84.4% versus 80.9%, $P=0.04$). However, subgroup analysis demonstrated that the survival benefit seen with CABG was confined to diabetic patients treated with insulin or oral hypoglycemic agents.

Conclusions

In general, the indications for PCI and CABG in UA/NSTEMI are similar to those in stable angina. High-risk patients with LV systolic dysfunction, 2-vessel disease with severe proximal LAD involvement, severe 3-vessel disease, or left main disease should be considered for CABG. Many other patients will have less severe CAD that does not put them at high risk for cardiac death. However, even less severe disease can have a substantial negative affect on the quality of life. Compared with high-risk patients, low-risk patients receive negligible or very modestly increased chances of long-term survival with CABG. Therefore, in low-risk patients, quality of life and patient preferences are given more

weight than are strict clinical outcomes in the selection of a treatment strategy. Low-risk patients whose symptoms do not respond well to maximal medical therapy and who experience a significant negative affect on their quality of life and functional status should be considered for revascularization.

V. Hospital Discharge and Post-Hospital Discharge Care

The acute phase of UA/NSTEMI is usually over within 2 months. The risk of progression to MI or the development of recurrent MI or death is highest during that period. At 1 to 3 months after the acute phase, most patients resume a clinical course similar to that of patients with chronic stable coronary disease.

A. Medical Regimen

An effort of the entire staff (physicians, nurses, dietitians, pharmacists, rehabilitation specialists, and physical and occupational therapists) is often necessary to prepare the patient for discharge. Direct patient instruction is important and should be reinforced and documented with written instruction sheets. Enrollment in a cardiac rehabilitation program after discharge may enhance patient education and enhance compliance with the medical regimen.

Recommendations for Postdischarge Therapy

Class I

1. Before hospital discharge, patients and/or designated responsible caregivers should be provided with well-understood instructions with respect to medication type, purpose, dose, frequency, and pertinent side effects. (Level of Evidence: C)
2. Drugs required in the hospital to control ischemia should be continued after hospital discharge in patients who do not undergo coronary revascularization, patients with unsuccessful revascularization, or patients with recurrent symptoms after revascularization. Upward or downward titration of the doses may be required. (Level of Evidence: C)
3. Before hospital discharge, patients should be informed about symptoms of acute myocardial infarction and should be instructed in how to seek help if they occur. (Level of Evidence: C)
4. All patients should be given sublingual or spray NTG and instructed in its use. (Level of Evidence: C)
5. Anginal discomfort that lasts >2 or 3 minutes should prompt the patient to discontinue the activity or remove himself or herself from the stressful event. If pain does not subside immediately, the patient should be instructed to take NTG. If the first tablet or spray does not provide relief within 5 minutes, then a second and third dose, at 5-minute intervals, should be taken. Pain that lasts >15 to 20 minutes or persistent pain despite 3 NTG doses should prompt the patient to seek immediate medical attention by calling 9-1-1 and going to the nearest hospital ED, preferably by ambulance or the quickest available alternative. (Level of Evidence: C)

6. If the pattern of anginal symptoms changes (eg, pain that is more frequent or severe, is precipitated by less effort, or now occurs at rest), the patient should contact his or her physician to determine the need for additional treatment or testing. (Level of Evidence: C)
7. ASA 75 to 325 mg/d in the absence of contraindications. (Level of Evidence: A)
8. Clopidogrel 75 mg/d in patients with a contraindication to ASA. (Level of Evidence: B)
9. β -Blockers in the absence of contraindications. (Level of Evidence: B)
10. Lipid-lowering agents and diet in post ACS patients including patients who are post revascularization with low-density lipoprotein (LDL) cholesterol of >125 mg/dL, including after revascularization. (Level of Evidence: A)
11. Lipid-lowering agents if LDL cholesterol level after diet is >100 mg/dL. (Level of Evidence: C)
12. ACEIs for patients with CHF, LV dysfunction (EF <0.40), hypertension, or diabetes. (Level of Evidence: A)

A reduction in the mortality and vascular event rates was reported in 1 large trial, the Heart Outcomes Prevention Evaluation (HOPE) Study, with the long-term use of an ACEI in moderate-risk patients with CAD, many of whom had preserved LV function, as well as in patients at a high risk of developing CAD. Although observational data suggest a protective effect of hormone replacement therapy (HRT) for coronary events, the only randomized trial of HRT for secondary prevention of death and MI that has been completed (Heart and Estrogen/progestin Replacement Study [HERS]) failed to demonstrate a beneficial effect. It is recommended that postmenopausal women on HRT continue but that HRT *not* be initiated for the secondary prevention of coronary events.

B. Postdischarge Follow-Up Recommendations

Class I

1. Discharge instructions should include a follow-up appointment. Low-risk medically treated patients and revascularized patients should return in 2 to 6 weeks, and higher-risk patients should return in 1 to 2 weeks. (Level of Evidence: C)
2. Patients managed initially with a conservative strategy who experience recurrent unstable angina or severe (Canadian Cardiovascular Society [CCS] Class III) chronic stable angina despite medical management and who are suitable for revascularization should undergo coronary arteriography. (Level of Evidence: B)
3. Patients who have tolerable stable angina or no anginal symptoms at follow-up visits should be managed with long-term medical therapy for stable CAD. (Level of Evidence: B)

C. Risk Factor Modification Recommendations

Class I

1. Specific instructions should be given regarding the following:

- a) Smoking cessation and achievement or maintenance of optimal weight, daily exercise, and diet (Level of Evidence: B)
 - b) Hypertension control to a blood pressure of <130/85 mm Hg (Level of Evidence: A)
 - c) Tight control of hyperglycemia in diabetes (Level of Evidence: B)
 - d) HMG-CoA reductase inhibitors for LDL cholesterol of >130 mg/dL. (Level of Evidence: C)
 - e) Lipid-lowering agent if LDL >100 mg/dL after diet. (Level of Evidence: B)
2. Consider the referral of patients who are smokers to a smoking cessation program or clinic and/or an outpatient cardiac rehabilitation program. (Level of Evidence: B)

Class IIa

1. Gemfibrozil or niacin in patients with a high-density lipoprotein (HDL) cholesterol level of <40 mg/dL and a triglyceride level of >200 mg/dL. (Level of Evidence: B)

There is a wealth of evidence that cholesterol-lowering therapy for patients with CAD and hypercholesterolemia and for patients with mild cholesterol elevation (mean 209 to 218 mg/dL) after MI and UA reduces vascular event and death rates.

The healthcare team should work with patients and their families to educate them regarding specific targets for cholesterol, blood pressure, and weight. The family may be able to further support the patient by also making changes in risk behavior (eg, cooking low-fat meals for the entire family, exercising together). This is particularly important when screening of family members reveals common risk factors, such as hyperlipidemia, hypertension, and obesity.

Recommendation

Class I

1. Beyond the instructions for daily exercise, patients require specific instruction on activities (eg, heavy lifting, climbing stairs, yard work, household activities) that are permissible and those that should be avoided. Specific mention should be made regarding when they can resume driving and return to work. (Level of Evidence: C)

VI. Special Groups

A. Women

Recommendation

Class I

1. Women with UA/NSTEMI should be managed in a manner similar to men. Specifically, women, like men with UA/NSTEMI, should receive ASA and indications for noninvasive and invasive testing, and the results of revascularization are similar. (Level of Evidence: B)

B. Diabetes Mellitus

Recommendations

Class I

1. Diabetes is an independent prognostic factor for increased risk, and this should be taken into account in the initial evaluation. (Level of Evidence: A)

2. Medical treatment in the acute phase and decisions on whether to perform stress testing and angiography and revascularization should be similar in diabetic and nondiabetic patients. (Level of Evidence: C)
3. Attention should be directed toward tight glucose control. (Level of Evidence: B)
4. For patients with multivessel disease, CABG with use of the internal mammary arteries is preferred over PCI in patients who are receiving treatment for diabetes. (Level of Evidence: B)

Class IIa

1. PCI for diabetic patients with 1-vessel disease and inducible ischemia. (Level of Evidence: B)
2. Abciximab for diabetics treated with coronary stenting. (Level of Evidence: B)

Diabetes occurs in about one fifth of patients with UA/NSTEMI and is an independent predictor of adverse outcomes. It is associated with more extensive CAD, unstable lesions, frequent comorbidities, and less favorable long-term outcomes with coronary revascularization, especially with PTCA. The use of stents, particularly with abciximab, appears to provide more favorable results in diabetics, although more data are needed. Clinical outcome with CABG, especially using 1 or both internal mammary arteries, is better than that with PTCA but is still less favorable than in nondiabetics.

C. Post-CABG Patients

Recommendations

Class I

1. Medical treatment in post-CABG patients should follow the same guidelines as for non-post-CABG patients with UA/NSTEMI. (Level of Evidence: C)
2. Because of the many anatomic possibilities that might be responsible for recurrent ischemia, there should be a low threshold for angiography in post-CABG patients with UA/NSTEMI. (Level of Evidence: B)

Class IIa

1. Repeat CABG for multiple SVG stenoses, especially when there is significant stenosis of a graft that supplies the LAD; PCI for focal saphenous vein stenosis. (Level of Evidence: C)
2. Stress testing should in general involve imaging in post-CABG patients. (Level of Evidence: C)

Overall, up to 20% of UA/NSTEMI patients are status post CABG. Conversely, ≈20% of post-CABG patients develop UA/NSTEMI over 7.5 years, with a highly variable postoperative time of occurrence. Post-CABG patients who present with UA/NSTEMI are at a higher risk with more extensive CAD and LV dysfunction than previously unoperated patients.

Post-CABG patients, especially those with only SVGs, are at a high risk of ACS and other adverse cardiac outcomes, including UA/NSTEMI. There is a high likelihood of disease in SVGs versus native arteries that increases with postoperative time. There are also difficulties with treadmill ECG testing and less favorable outcomes with repeat revascular-

ization than in patients who have not undergone previous CABG.

D. Elderly Patients

Recommendations

Class I

1. Decisions on management should reflect considerations of general health, comorbidities, cognitive status, and life expectancy. (Level of Evidence: C)
2. Attention should be paid to altered pharmacokinetics and sensitivity to hypotensive drugs. (Level of Evidence: B)
3. Intensive medical and interventional management of ACS may be undertaken but with close observation for adverse effects of these therapies. (Level of Evidence: B)

Elderly persons with UA/NSTEMI tend to have atypical presentations of disease, substantial comorbidity, ECG stress tests that are more difficult to interpret, and different responses to pharmacological agents compared with younger patients. Their outcomes with interventions and surgery are not as favorable as those of younger patients, in part because of greater comorbidities, but coronary revascularization can be performed when the same group of prognostic risk factors that play a role in the younger age group are taken into account. The approach to these patients also must include consideration of the general medical and mental status and the anticipated life expectancy. Very frail elderly patients represent a high-risk group and should be evaluated for revascularization on a case-by-case basis. In many of these patients, even those with diffuse coronary arterial disease, PCI, with its lower morbidity rates, may be preferable to CABG.

E. Cocaine

Recommendations

Class I

1. NTG and oral calcium antagonists for patients with ST-segment elevation or depression that accompanies ischemic chest discomfort. (Level of Evidence: C)
2. Immediate coronary arteriography, if possible, in patients whose ST segments remain elevated after NTG and calcium antagonists; thrombolysis (with or without PCI) if thrombus is detected. (Level of Evidence: C)

Class IIa

1. Intravenous calcium antagonists for patients with ST-segment deviation suggestive of ischemia. (Level of Evidence: C)
2. β -Blockers for hypertensive patients (systolic blood pressure >150 mm Hg) or those with sinus tachycardia (pulse >100 bpm). (Level of Evidence: C)
3. Thrombolytic therapy if ST segments remain elevated despite NTG and calcium antagonists and coronary arteriography is not possible. (Level of Evidence: C)

4. Coronary arteriography, if available, for patients who have ST-segment depression or isolated T-wave changes not known to be old and who are unresponsive to NTG and calcium antagonists. (Level of Evidence: C)

Class III

1. Coronary arteriography in patients with chest pain without ST-T-wave changes. (Level of Evidence: C)

The basis for cocaine-induced coronary spasm has been demonstrated in both in vitro and in vivo experiments in animals and humans. The use of cocaine is associated with a number of cardiac complications that can produce myocardial ischemia, and cocaine users may develop ischemic chest discomfort that is indistinguishable from UA/NSTEMI. The widespread use of cocaine makes it mandatory to consider this cause, because its recognition mandates special management.

F. Variant (Prinzmetal's) Angina

Recommendations

Class I

1. Coronary arteriography in patients with episodic chest pain and ST-segment elevation that resolves with NTG and/or calcium antagonists. (Level of Evidence: B)
2. Treatment with nitrates and calcium antagonists in patients whose coronary arteriogram is normal or shows only nonobstructive lesions. (Level of Evidence: B)

Class IIa

1. Provocative testing in patients with a nonobstructive lesion on coronary arteriography, the clinical picture of coronary spasm, and transient ST-segment elevation. (Level of Evidence: B)

Class IIb

1. Provocative testing without coronary arteriography. (Level of Evidence: C)
2. In the absence of significant CAD on coronary arteriography, provocative testing with methylergonovine, acetylcholine, or methacholine when coronary spasm is suspected but there is no ECG evidence of transient ST-segment elevation. (Level of Evidence: C)

Class III

1. Provocative testing in patients with high-grade obstructive lesions on coronary arteriography. (Level of Evidence: B)

Variant (Prinzmetal's) angina is a form of UA that usually occurs spontaneously, is characterized by transient ST-segment elevation, and most commonly resolves without progression to MI. The earliest stages of AMI may also be associated with cyclic ST-segment elevations. It is caused by coronary spasm that is most commonly focal and can occur simultaneously at >1 site.

Coronary spasm is usually very responsive to NTG, long-acting nitrates, and calcium antagonists. Smoking should be

discontinued. Usually, a calcium antagonist at a high dose (verapamil 240 to 480 mg/d, diltiazem 120 to 360 mg/d, nifedipine 60 to 120 mg/d) is started. If the episodes are not completely eliminated, a second calcium antagonist from another class or a long-acting nitrate should be added. α -Receptor blockers have also been reported to be of benefit, especially in patients who are not responding completely to calcium antagonists and nitrates.

Recommendations for Patients With Syndrome X

Class I

1. Reassurance and medical therapy with nitrates, β -blockers, and calcium antagonists alone or in combination. (Level of Evidence: B)
2. Risk factor reduction. (Level of Evidence: C)

Class IIb

1. Intracoronary ultrasound to rule out missed obstructive lesions. (Level of Evidence: B)
2. If no ECGs are available during chest pain and coronary spasm cannot be ruled out, coronary arteriography and provocative testing with methylergonovine, acetylcholine, or methacholine. (Level of Evidence: C)
3. HRT in postmenopausal women unless there is a contraindication. (Level of Evidence: C)

4. Imipramine for continued pain despite Class I measures. (Level of Evidence: C)

Class III

1. Medical therapy with nitrates, β -blockers, and calcium antagonists for patients with noncardiac chest pain. (Level of Evidence: C)

The term "syndrome X" is used to describe patients with angina or angina-like discomfort with exercise, ST-segment depression on treadmill testing, and normal or nonobstructed coronary arteries on arteriography. Syndrome X is more common in women than in men. Chest pain can vary from that of typical angina pectoris to chest pain with atypical features to chest pain that simulates UA, secondary to CAD. The intermediate-term prognosis of patients with syndrome X is excellent.

It is recommended that patients be reassured of the excellent intermediate-term prognosis and be treated with long-acting nitrates. If the patient continues to have episodes of chest pain, a calcium antagonist or β -blocker can be started. Imipramine at 50 mg HS has been successful in reducing the frequency of chest pain episodes.

KEY WORDS: ACC/AHA Practice Guidelines ■ angina ■ diagnosis

Correction

In the “ACC/AHA Guidelines for the Management of Patients With Unstable Angina and Non-ST-Segment Elevation Myocardial Infarction: Executive Summary and Recommendations” by Braunwald et al that appeared in a previous issue of the journal (*Circulation*. 2000;102:1193–1209), the following errors need to be corrected.

On Page 1195 in Table 1 in the “Low Risk” column, the first entry should read: “New-onset or progressive CCS Class III or IV angina in the past 2 weeks without prolonged (>20 min) rest pain but with moderate or high likelihood of CAD.”

In the same Table under the “High Risk” column. “Cardiac markers” row, in the first line of the entry, delete “markedly” to read: “Elevated (eg, TnT or TnI >0.1 ng/mL).”

The correct table follows.

On page 1201 in the first column under Recommendation 4, 4th line, “(see Table 2)” should be replaced with “(see Table 1).” The recommendation should read:

4. A platelet GP IIb/IIIa receptor antagonist should be administered, in addition to ASA and UFH, to patients with continuing ischemia or with other high-risk features (see Table 1) and to patients in whom a percutaneous coronary intervention (PCI) is planned. Eptifibatide and tirofiban are approved for this use. (Level of Evidence: A) Abciximab can also be used for 12 to 24 hours in patients with UA/NSTEMI in whom a PCI is planned within the next 24 hours. (Level of Evidence: A)

On page 1203 in Figure 4 in “All” graph on the left, “3.9%” should be changed to “2.9%.” The corrected figure follows.

On page 1206 under “Recommendations for Postdischarge Therapy,” change >125 mg/dL to >130 mg/dL in item 10. The recommendation should read:

10. Lipid-lowering agents and diet in post ACS patients including patients who are post revascularization with low-density lipoprotein (LDL) cholesterol of >130 mg/dL, including after revascularization. (Level of Evidence: A)

TABLE 1. Short-Term Risk of Death or Nonfatal MI in Patients With UA

Feature	High Risk (At least 1 of the following features must be present)	Intermediate Risk (No high-risk feature but must have 1 of the following features)	Low Risk (No high- or intermediate-risk feature but may have any of the following features)
History	Accelerating tempo of ischemic symptoms in preceding 48 hrs	Prior MI, peripheral or cerebrovascular disease, or CABG; prior aspirin use	
Character of pain	Prolonged ongoing (>20 min) rest pain	Prolonged (>20 min) rest angina, now resolved, with moderate or high likelihood of CAD Rest angina (<20 min or relieved with rest or sublingual NTG)	New-onset or progressive CCS Class III or IV angina in the past 2 wk without prolonged (>20 min) rest pain but with moderate or high likelihood of CAD
Clinical findings	Pulmonary edema, most likely related to ischemia New or worsening MR murmur S ₃ or new/worsening rales Hypotension, bradycardia, tachycardia Age >75 y	Age >70 y	
ECG findings	Angina at rest with transient ST-segment changes >0.05 mV Bundle-branch block, new or presumed new Sustained ventricular tachycardia	T-wave inversions >0.2 mV Pathological Q waves	Normal or unchanged ECG during an episode of chest discomfort
Cardiac markers	Elevated (eg, TnT or TnI >0.1 ng/mL)	Slightly elevated (eg, TnT >0.01 but <0.1 ng/mL)	Normal

An estimation of the short-term risks of death and nonfatal cardiac ischemic events in UA is a complex multivariable problem that cannot be fully specified in a table such as this. Therefore, the table is meant to offer general guidance and illustration rather than rigid algorithms.

Adapted with permission from Braunwald E, Mark DB, Jones RH, et al. Unstable angina: diagnosis and management. Rockville, MD: Agency for Health Care Policy and Research and the National Heart, Lung, and Blood Institute, US Public Health Service, US Department of Health and Human Services; 1994; AHCPR Publication No. 94-0602. AHCPR Clinical Practice Guideline No. 10, Unstable Angina: Diagnosis and Management, May 1994.

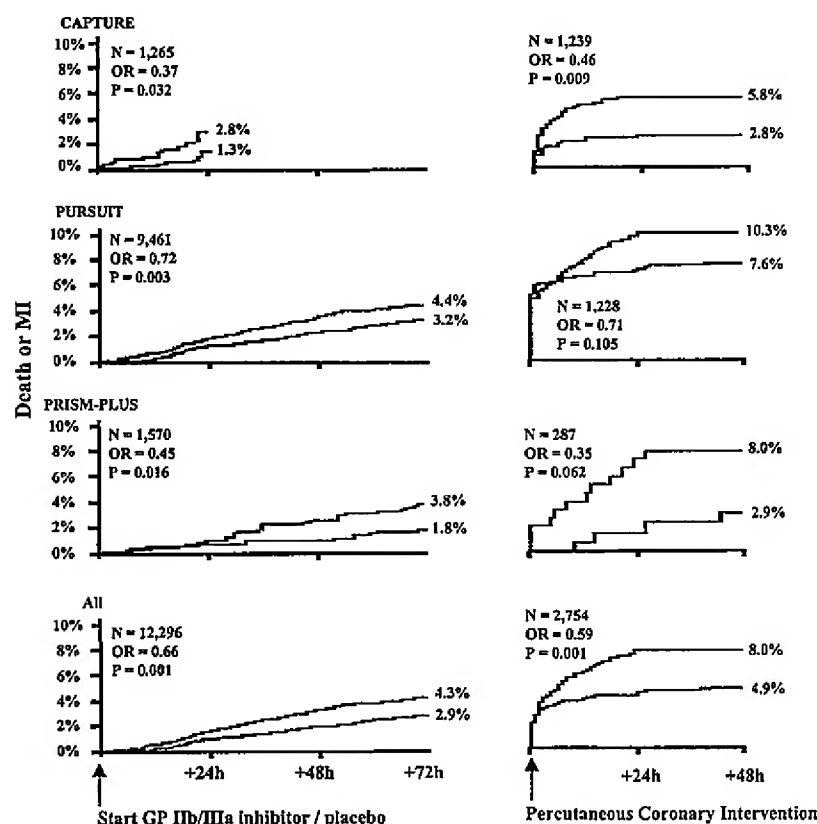


Figure 4. Kaplan-Meier curves showing cumulative incidence of death or MI in patients randomly assigned to platelet GP IIb/IIIa receptor antagonist (bold line) or placebo. Data are derived from the CAPTURE, PURSUIT, and PRISM-PLUS trials. Left, Events during the initial period of medical treatment until the moment of PCI or CABG. In the CAPTURE trial, abciximab was administered for 18 to 24 hours before the PCI was performed in almost all patients as per study design; abciximab was discontinued 1 hour after the intervention. In PURSUIT, a PCI was performed in 11.2% of patients during a period of medical therapy with eptifibatide that lasted 72 hours and for 24 hours after the intervention. In PRISM-PLUS, an intervention was performed in 30.2% of patients after a 48-hour period of medical therapy with tirofiban, and the drug infusion was maintained for 12 to 24 hours after an intervention. Right, Events occurring at the time of PCI and the next 48 hours, with the event rates reset to 0% before the intervention. CK or CK-MB elevations exceeding 2 times the upper limit of normal were considered as infarction during medical management and exceeding 3 times the upper limit of normal for PCI-related events. OR indicates odds ratio. Adapted with permission from Boersma E, Akkerhuis KM, Theroux P, et al. Platelet glycoprotein IIb/IIIa receptor inhibition in non-ST-elevation acute coronary syndromes: early benefit during medical treatment only, with additional protection during percutaneous coronary intervention. *Circulation*. 1999;100:2045-2048.

ACC/AHA Guidelines for the Management of Patients With Unstable Angina and Non-ST-Segment Elevation Myocardial Infarction: Executive Summary and Recommendations

A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on the Management of Patients With Unstable Angina)

An incorrect sentence (The present guidelines supersede the 1994 guidelines.) on the first page of the guidelines (*Circulation*. 2000;102:1193–1209.) was printed. We regret the error and provide the correct text below in brackets.

This Task Force therefore formed the current committee to develop guidelines for the management of unstable angina and non-ST-segment elevation myocardial infarction, [supported by the Agency for Healthcare Research and Quality's UCSF-Stanford Evidence-Based Practice Center. This document will serve as a useful successor to the 1994 AHCPR guidelines.]

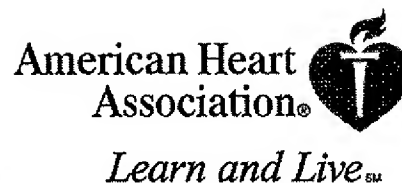
Additionally, information regarding endorsement of the guidelines was omitted from publication. These guidelines have been officially endorsed by the American College of Emergency Physicians (ACEP)* and the Society for Cardiac Angiography and Interventions.

*Endorsement by ACEP means that ACEP agrees with the general concepts in the guidelines and believes that the developers have begun to define a process of care that considers the best interests of patients with unstable angina and non-ST-segment elevation myocardial infarction.

Exhibit 9

Circulation

JOURNAL OF THE AMERICAN HEART ASSOCIATION



ACC/AHA Guidelines for Percutaneous Coronary Intervention (Revision of the 1993 PTCA Guidelines)—Executive Summary : A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1993 Guidelines for Percutaneous Transluminal Coronary Angioplasty)

Endorsed by the Society for Cardiac Angiography and Interventions

Sidney C. Smith, Jr, James T. Dove, Alice K. Jacobs, J. Ward Kennedy, Dean Kereiakes, Morton J. Kern, Richard E. Kuntz, Jeffery J. Popma, Hartzell V. Schaff, David O. Williams, Raymond J. Gibbons, Joseph P. Alpert, Kim A. Eagle, David P. Faxon, Valentin Fuster, Timothy J. Gardner, Gabriel Gregoratos, Richard O. Russell and Sidney C. Smith, Jr

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ACC/AHA Practice Guidelines

ACC/AHA Guidelines for Percutaneous Coronary Intervention (Revision of the 1993 PTCA Guidelines)—Executive Summary

A Report of the American College of Cardiology/American Heart
Association Task Force on Practice Guidelines (Committee to Revise
the 1993 Guidelines for Percutaneous Transluminal Coronary Angioplasty)

Endorsed by the Society for Cardiac Angiography and Interventions

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I. Introduction

The American College of Cardiology/American Heart Association (ACC/AHA) Task Force on Practice Guidelines was formed to gather information and make recommendations about appropriate use of technology for the diagnosis and treatment of patients with cardiovascular disease. Percutaneous coronary interventions (PCI) are an important group of technologies in this regard. Although initially limited to PTCA, and termed percutaneous transluminal coronary angioplasty (PTCA), PCI now includes other new techniques capable of relieving coronary narrowing. Accordingly, in this document, rotational atherectomy, directional atherectomy, extraction atherectomy, laser angioplasty, implantation of intracoronary stents and other catheter devices for treating coronary atherosclerosis are considered components of PCI. In this context PTCA will be used to refer to those studies using primarily PTCA while PCI will refer to the broader group of percutaneous techniques. These new technologies have impacted the effectiveness and safety profile initially

established for PTCA. Moreover, important advances have occurred in the use of adjunctive medical therapies such as glycoprotein (GP) IIb/IIIa receptor blockers. In addition, since publication of the previous Guidelines in 1993, greater experience in the performance of PCI in patients with acute coronary syndromes and in community hospital settings has been gained. In view of these developments, further review and revision of the guidelines is warranted. This document reflects the opinion of the third ACC/AHA committee charged with revising the guidelines for PTCA to include the broader group of technologies now termed PCI.

Several issues relevant to the Committee's process and the interpretation of the Guidelines have been noted previously and are worthy of restatement. First, PCI is a technique that has been continually refined and modified; hence continued, periodic Guideline revision is anticipated. Second, these guidelines are to be viewed as broad recommendations to aid in the appropriate application of PCI. Under unique circumstances, exceptions may exist. These Guidelines are intended

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A single reprint of this document is available by calling 800-242-8721 (US only) or by writing the American Heart Association, Public Information, 7272 Greenville Ave, Dallas, TX 75231-4596. Ask for reprint No. 71-0205. This document and the companion full-text guideline (reprint no. 71-0206) are available on the ACC Web site at www.acc.org and the AHA Web site at www.americanheart.org. To purchase additional reprints (specify version): up to 999 copies, call 800-611-6083 (US only) or fax 413-665-2671; 1000 or more copies, call 214-706-1466, fax 214-691-6342; or e-mail: pubauth@heart.org (*Circulation* 2001;103:3019-3041.)

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to complement, not replace, sound medical judgment and knowledge. They are intended for operators who possess the cognitive and technical skills for performing PCI and assume that facilities and resources required to properly perform PCI are available. As in the past, the indications are categorized as Class I, II, or III based on a multifactorial assessment of risk as well as expected efficacy viewed in the context of current knowledge and the relative strength of this knowledge. Initially, this document describes the background information that forms the foundation for specific indications. Topics fundamental to coronary intervention are reviewed followed by separate discussions relating to unique technical and operational issues. **Formal recommendations for the use of angioplasty are included in Section V.** Indications are organized according to clinical presentation. This format is designed to enhance the usefulness of this document for the assessment and care of patients with coronary artery disease (CAD).

This document employs the ACC/AHA style classification as Class I, II, or III. These classes summarize the indications for PCI as follows:

- Class I:** Conditions for which there is evidence for and/or general agreement that the procedure or treatment is useful and effective.
- Class II:** Conditions for which there is conflicting evidence and/or a divergence of opinion about the usefulness/efficacy of a procedure or treatment.
 - Class IIa:** Weight of evidence/opinion is in favor of usefulness/efficacy.
 - Class IIb:** Usefulness/efficacy is less well established by evidence/opinion.
- Class III:** Conditions for which there is evidence and/or general agreement that the procedure/treatment is not useful/effective, and in some cases may be harmful.

The weight of evidence in support of the recommendation for each listed indication is presented as follows:

- Level of Evidence A:** Data derived from multiple randomized clinical trials.
- Level of Evidence B:** Data derived from a single randomized trial or nonrandomized studies.
- Level of Evidence C:** Consensus opinion of experts.

The ACC/AHA Task Force on Practice Guidelines makes every effort to avoid any actual or potential conflicts of interest that might arise as a result of an outside relationship or personal interest of a member of the writing panel. Specifically, all members of the writing panel are asked to provide disclosure statements of all such relationships that might be perceived as real or potential conflicts of interest. These statements are reviewed by the parent task force, reported orally to all members of the writing panel at the first meeting, and updated as changes occur.

II. General Considerations and Background

More than 500,000 PCI procedures are performed yearly in the U.S., and it has been estimated that more than 1,000,000 procedures are performed annually worldwide. New coronary devices have expanded the clinical and anatomical indications for revascularization initially limited by balloon catheter angioplasty. For example, stents reduce both the acute risk of major complications and late-term restenosis. The success of new coronary devices in meeting these goals is in part represented by the less frequent use of PTCA alone (<30%) and the high (>70%) penetration of coronary stenting in the current practice of interventional cardiology. Atherectomy devices and stenting, associated with improved acute angiographic and clinical outcomes compared to PTCA, in specific subsets, continue to be applied to a wider patient domain that includes multivessel disease and complex coronary anatomy. However, strong evidence (level A data from multiple randomized clinical trials) is only available for stenting in selected patients undergoing single-vessel PCI. These Guidelines will focus on the Food and Drug Administration (FDA) approved balloon-related and nonballoon coronary revascularization devices.

III. Outcomes

The outcomes of coronary interventional procedures are measured in terms of success and complications and are related to the mechanisms of the employed devices, as well as the clinical and anatomic patient-related factors. With increased operator experience, new technology, and adjunctive pharmacotherapy, the overall success and complication rates of angioplasty have improved.

A. Definitions of PCI Success

The success of a PCI procedure may be defined by angiographic, procedural, and clinical criteria.

1. Angiographic Success

A successful PCI produces substantial enlargement of the lumen at the target site. The consensus definition prior to the widespread use of stents was the achievement of a minimum stenosis diameter reduction to <50% in the presence of grade 3 TIMI flow (assessed by angiography). However, with the advent of advanced adjunct technology, including coronary stents, a minimum stenosis diameter reduction to <20% has been the clinical benchmark of an optimal angiographic result.

2. Procedural Success

A successful PCI should achieve angiographic success without in-hospital major clinical complications (e.g., death, myocardial infarction [MI], emergency coronary artery bypass surgery [CABG]) during hospitalization. Although the occurrence of emergency artery coronary bypass surgery and death are easily identified end points, the definition of procedure-related MI has been debated. The development of Q-waves in addition to a threshold value of CK elevation has been commonly used. However, the significance of enzyme elevations in the absence of Q-waves remains a subject of investigation and debate. Several reports have identified non-Q-wave MIs with CK-MB elevations 3 to 5 times the

upper limit of normal as having clinical significance. Thus a significant increase in CK-MB without Q-waves is considered by most to qualify as an associated complication of PCI.

If serial determinations are performed after PCI, an abnormally high value (CK-MB >1 times normal) can be expected in 10 to 15% of PTCA procedures, 15 to 20% of stent procedures, 25 to 35% of atherectomy procedures, and >25% for any device used in saphenous vein grafts (SVGs) or long lesions with a high atherosclerotic burden, even in the absence of other signs and symptoms of MI. There is no accepted consensus on what level of CK-MB index (with or without clinical or electrocardiographic [ECG] findings) is indicative of a clinically important MI following the interventional procedure. Cardiac troponin T and I have now been introduced as measurements of myocardial necrosis and have been proven to be more sensitive and specific than CK-MB. However, prognostic criteria after PCI based on troponin T and I have not yet been developed. The Writing Committee recommends that CK-MB determination be performed on all patients who have signs or symptoms suggestive of MI following the procedure or in patients in whom there is angiographic evidence of abrupt vessel closure, important side branch occlusion, or new and persistent slow coronary flow. In patients in whom a clinically driven CK-MB determination is made, a CK-MB of >3 times the upper limit of normal would constitute a clinically significant MI.

3. Clinical Success

In the short term, a clinically successful PCI includes anatomic and procedural success with relief of signs and/or symptoms of myocardial ischemia after the patient recovers from the procedure. The long-term clinical success requires that the short-term clinical success remains durable and that the patient has persistent relief of signs and symptoms of myocardial ischemia for more than 6 months after the procedure. Restenosis is the principal cause of lack of long-term clinical success when a short-term clinical success has been achieved.

B. Definitions of Procedural Complications

As outlined in the 1998 coronary interventional document, procedural complications are divided into six basic categories: death, MI, emergency CABG, stroke, vascular access site complications, and contrast agent nephropathy. Key data elements and definitions to measure the clinical management and outcomes of patients undergoing diagnostic catheterization and/or PCI have been defined in the Clinical Data Standards document and the ACC-National Cardiovascular Data Registry™ Catheterization Laboratory Module version 2.0. These rigorous definitions for key adverse events are endorsed by this Writing Committee for inclusion in the present PCI Guidelines (Table 1).

C. Acute Outcome

Improvements in balloon technology coupled with the increased use of nonballoon devices, particularly stents (which are effective in treating abrupt vessel closure) and GP IIb/IIIa platelet receptor antagonists have favorably influenced acute procedural outcome. This combined balloon/device/pharma-

cologic approach to coronary intervention in elective procedures has resulted in angiographic success rates of 96 to 99%, with Q-wave MI rates of 1 to 3%, emergency coronary bypass surgery rates of 0.2 to 3%, and unadjusted in-hospital mortality rates of 0.5 to 1.4%.

D. Long-Term Outcome and Restenosis

Although improvements in technology, including stents and new pharmacologic therapy, have resulted in an improved acute outcome of the procedure, the impact of these changes on long-term (5 to 10 years) outcome may be less dramatic where factors such as advanced age, reduced left ventricular (LV) function, and complex multivessel disease in patients currently undergoing PCI may have a more important influence. In addition, available data on long-term outcome are mostly limited to patients undergoing PTCA. Ten-year follow-up of the initial cohort of patients treated with PTCA revealed an 89.5% survival rate (95% in patients with single-vessel disease, 81% in patients with multivessel disease). In patients within the 1985–1986 NHLBI PTCA Registry, 5-year survival was 92.9% for patients with single-vessel disease, 88.5% for those with 2-vessel disease, and 86.5% for those with 3-vessel disease. In patients with multivessel disease undergoing PTCA in BARI, 5-year survival was 86.3% and infarct-free survival was 78.7%. Specifically, 5-year survival was 84.7% in patients with 3-vessel disease and 87.6% in patients with 2-vessel disease.

In addition to the presence of multivessel disease, other clinical factors adversely impact late mortality. In randomized patients with treated diabetes in BARI, the 5-year survival was 65.5%, and the cardiac mortality was 20.6% in comparison to 5.8% cardiac mortality in patients without treated diabetes, although among eligible but not randomized diabetic patients, the 5-year cardiac mortality was 7.5%. In the 1985–1986 NHLBI PTCA Registry, 4-year survival was significantly lower in women (89.2%) in comparison to men (93.4%). In addition, although LV dysfunction was not associated with an increase in in-hospital mortality or nonfatal MI in patients undergoing PTCA in the same registry, it was an independent predictor of a higher long-term mortality.

A major determinant of event-free survival following coronary intervention is the incidence of restenosis which had, until the development of stents, remained fairly constant, despite multiple pharmacologic and mechanical approaches to limit this process (Table 2). Depending on the definition, (i.e., whether clinical or angiographic restenosis or target lesion revascularization is measured), the incidence of restenosis following coronary intervention had been 30 to 40%, and higher in certain clinical and angiographic subsets.

Although multiple clinical factors (diabetes, unstable angina, acute MI, prior restenosis), angiographic factors (proximal left anterior descending artery, small vessel diameters, total occlusion, long lesion length, SVG), and procedural factors (higher post-procedure percent diameter stenosis, smaller minimal lumen diameter, and smaller acute gain), have been associated with an increased incidence of restenosis, the ability to integrate these factors and predict the risk of restenosis in individual patients following the procedure remains difficult. The most prom-

TABLE 1. Definitions of Procedural Complications

Procedural Complications	Definitions
Primary cause of death	Patient died during this hospitalization
Periprocedural MI	The NEW presence of an MI as documented by at least 1 of the following criteria: 1. Evolutionary ST-segment elevations, development of new Q-waves in 2 or more contiguous ECG leads, or new or presumably new LBBB pattern on the ECG 2. Biochemical evidence of myocardial necrosis; this can be manifested as 1) CK-MB $\geq 3 \times$ the upper limit of normal or if CK-MB not available (2) total CK $\geq 3 \times$ upper limit of normal. Because normal limits of certain blood tests may vary, please check with your lab for normal limits for CK-MB and total CK
CABG during this admission	If the patient had a CABG during this admission indicate the CABG status using the following categories: I. Elective: The procedure could be deferred without increased risk of compromised cardiac outcome II. Urgent: All of the following conditions are met: A. Not elective B. Not emergency C. Procedure required during same hospitalization in order to minimize chance of further clinical deterioration III. Emergency: The patient's clinical status includes any of the following: A. Ischemic dysfunction (any of the following): 1. Ongoing ischemia including rest angina despite maximal medical therapy (medical and/or IABP) 2. Acute evolving MI within 24 hours before intervention 3. Pulmonary edema requiring intubation B. Mechanical dysfunction (either of the following): 1. Shock with circulatory support 2. Shock without circulatory support IV. Salvage: The patient is undergoing CPR en route to the Operating Room
CVA/Stroke	Patient experienced a cerebrovascular accident (CVA) as documented by a loss of neurological function caused by an ischemic event with residual symptoms at least 24 hours after onset
Vascular complications	
Bleeding	Blood loss at the site of arterial or venous access or due to perforation of a traversed artery or vein requiring transfusion and/or prolonging the hospital stay, and/or causing a drop in hemoglobin >3.0 gm/dl. Bleeding attributable to the vascular site could be retroperitoneal, a local hematoma >10 cm diameter or external
Occlusion	A total obstruction of the artery usually at the site of access requiring surgical repair. Occlusion is defined as total obstruction of the artery by thrombus, dissection or other mechanism, usually at the site of access, requiring surgical repair. Occlusion may be accompanied by absence of palpable pulse or Doppler signal and associated with signs and symptoms of an ischemic limb requiring surgical intervention
Dissection	A dissection occurred at the site of percutaneous entry. Dissection is defined as disruption of an arterial wall resulting in splitting and separation of the intimal (or subintimal) layers
Pseudoaneurysm	Pseudoaneurysm is defined as the occurrence of an aneurysmal dilatation of the artery at the site of catheter entry demonstrated by arteriography or ultrasound
AV fistula	AV fistula is defined as a connection between the access artery (e.g., femoral) and access vein (e.g., femoral) that is demonstrated by an imaging study (arteriography or ultrasound) and most often characterized by a continuous bruit
Renal failure	After the lab visit—but before any subsequent lab visits only: Indicate if the patient experienced acute renal insufficiency resulting in an increase in serum creatinine to more than 2.0 mg/dl (or a 50% or greater increase over an abnormal baseline) measured prior to procedure, or requiring dialysis

CABG = coronary artery bypass graft; CK = creatine kinase; CPR = cardiopulmonary resuscitation; ECG = electrocardiographic; IABP = intra-aortic balloon pump; LBBB = left bundle-branch block; MI = myocardial infarction.

using potential approaches to favorably impact the restenosis process relate to: 1) the ability to decrease elastic recoil and remodeling using intracoronary stents, and 2) to the ability to reduce intimal hyperplasia using catheter-based ionizing radiation. More than 6,300 patients have been studied in 12 randomized clinical trials to assess the efficacy of PTCA vs. stents to reduce restenosis (Table 3).

In addition, randomized studies in patients with in-stent restenosis have shown that both intracoronary gamma and beta radiation significantly reduced the rate of subsequent angiographic and clinical restenosis by 30 to 50%.

E. Predictors of Success/Complications

1. Anatomic Factors

The risk of PTCA in the pre-stent era relative to anatomic subsets has been identified in previous NHLBI PTCA Registry data and by the ACC/AHA Task Force. The lesion classification based on severity of characteristics proposed in the past has been principally altered using the present PCI techniques, which capitalize on the ability of stents to manage initial and subsequent complications of coronary interventions. As a result the Committee has revised the previous ACC/AHA lesion classification system to reflect low, moderate, and high risk (Table 4) in

TABLE 2. Selected Trials of Pharmacologic and Mechanical Approaches to Limit Restenosis

Study	Year	N	Agent	Restenosis Rate (%)	
				Placebo or Control	Agent
Schwartz	1988	376	Aspirin and Dipyridamole	39	38
Ellis	1989	416	Heparin	37	41
Pepine	1990	915	Methylprednisolone	39	40
CARPORT	1991	649	Vapiprost	19	21
O'Keefe	1991	197	Colchicine	22	22
MERCATOR	1992	735	Cilazapril	28	28
CAVEAT*	1993	500	DCA vs. PTCA	57	50
CCAT	1993	136	DCA vs. PTCA	43	46
Serruys	1993	658	Ketanserin	32	32
BENESTENT*	1994	520	Stent vs. PTCA	32	22
ERA	1994	458	Enoxaparin	51	52
Leaf	1994	551	Fish Oil	46	52
STRESS*	1994	410	Stent vs. PTCA	42	32
Weintraub	1994	404	Lovastatin	42	39
BOAT*	1996	492	DCA vs. PTCA	40	31
Wantanabe*	1996	118	Probucol	40	20
Tardif*	1997	317	Probucol	39	21
BENESTENT II*	1998	823	Stent vs. PTCA	31	17
TREAT*	1999	255	Tranilast	39	18
PRESTO*	2000	192	DCA and Tranilast	26	11

* $p < 0.05$.

DCA = Directional Coronary Atherectomy; PTCA = percutaneous transluminal coronary angioplasty.

accordance with the PCI Clinical Data Standards from the ACC-National Cardiovascular Data Registry™.

2. Clinical Factors

Coexistent clinical conditions can increase the complication rates for any given anatomic risk factor. The clinical risk factors associated with in-hospital adverse events have been further evaluated with additional experience during the PCI

era and summarized based on odds ratio >2.0 or results of multivariate analysis (Table 5).

3. Risk of Death

In the majority of patients undergoing elective PCI, death as a result of PCI is directly related to the occurrence of coronary artery occlusion and is most frequently associated with pro-

TABLE 3. Studies Comparing Balloon Angioplasty With Stents for Native Coronary Artery Lesions

Study	Year	Follow-Up, Month	N, Stent/Angioplasty	Angiographic Restenosis, %			Target-Vessel Revascularization (TVR), %			Death, MI, or TVR, %	
				Stent	Angioplasty	p Value	Stent	Angioplasty	p Value	Stent	Angioplasty
STRESS	1994	6	205/202	31.6	42.1	0.046	10.2	15.4	0.06	19.5	23.8
BENESTENT*	1996	12	259/257	—	—	—	10	21	0.001	23.2	31.5
TASC I	1995	6	270 (Overall)†	31	46	0.01	—	—	—	—	—
Versaci et al.	1997	12	60/60	19	40	0.02	6.6	22	—	—	—
STRESS II	1998	12	100/89	—	—	—	10	20	—	17	34
BENESTENT II	1998	6	413/410	16	31	<0.001	8‡	13.7	0.02	12.8	19.3
OCBAS	1998	7	57/59	18.8	16.6	—	17.5	9.2	—	19.2	16.9
EPISTENT§	1998	6	1603/796	—	—	—	8.7	15.4	<0.001	13	20.5
START	1999	6/48	229/223	22	37	<0.002	12	24.6	<0.002	16.9	29.9
OPUS	2000	6	479 (Overall)	—	—	—	3.0	10.1	0.003	6.1	14.9

*Any event at one year; †122 patients in the TASC I trial had treated restenotic lesions; ‡any repeat procedure; §stent plus abciximab vs. percutaneous transluminal coronary angioplasty plus abciximab; ||6 months angiographic follow-up and 48 months clinical follow-up.

MI = myocardial infarction; dashes (—) = data not reported for that category. Data are for lesions in coronary arteries with vessel diameter ≥ 3.0 mm. Adapted from Suwaidi MB, et al. JAMA 2000;284:1828–36.

TABLE 4. Lesion Classification System

Anatomic Risk Groups*
2000
PCI Stent Era
Low Risk
Discrete (length <10 mm)
Concentric
Readily accessible
Nonangulated segment (<45°)
Smooth contour
Little or no calcification
Less than totally occlusive
Not ostial in location
No major side branch involvement
Absence of thrombus
Moderate Risk
Tubular (length 10–20 mm)
Eccentric
Moderate tortuosity of proximal segment
Moderately angulated segment (>45°, <90°)
Irregular contour
Moderate or heavy calcification
Total occlusions <3 months old
Ostial in location
Bifurcation lesions requiring double guidewires
Some thrombus present
High Risk
Diffuse (length >20 mm)
Excessive tortuosity of proximal segment
Extremely angulated segments >90°
Total occlusions >3 months old and/or bridging collaterals
Inability to protect major side branches
Degenerated vein grafts with friable lesions

*This classification of lesion risk is cited from the ACC-National Cardiovascular Data Registry™ Catheterization Laboratory Module version 2.0. This classification scheme is also cited in the ACC Clinical Data Standards.

PCI = percutaneous coronary interventions.

nounced LV failure. The clinical and angiographic variables associated with increased mortality include advanced age, female gender, diabetes, prior MI, multivessel disease, left main or equivalent coronary disease, a large area of myocardium at risk, pre-existing impairment of LV or renal function, and collateral vessels supplying significant areas of myocardium that originate distal to the segment to be dilated (Table 5).

4. Women

In comparison to men, women undergoing PCI are older and have a higher incidence of hypertension, diabetes mellitus, hypercholesterolemia, and comorbid disease. Early reports of patients undergoing PTCA revealed a lower procedural success rate in women; however, more recent studies have noted similar angiographic outcome and incidence of MI and emergency coronary bypass surgery in women and men.

Although reports have been inconsistent, in several large-scale registries, in-hospital mortality is significantly higher in women and an independent effect of gender on acute mortality following PCI persists after adjustments for the baseline higher-risk profile in women.

5. The Elderly Patient

Age >75 years is one of the major clinical variables associated with increased risk of complications. In the elderly population, the morphologic and clinical variables are compounded by advanced years with the very elderly having the highest-risk of adverse outcomes. In the stent era, procedural success rates and short-term outcomes are comparable to those for nonoctogenarians. Thus, with rare exception (primary PCI for cardiogenic shock for patients >75 years), a separate category has not been created in these Guidelines for the elderly. However, their higher incidence of comorbidities should be taken into account when considering the need for PCI.

6. Diabetes Mellitus

In the TIMI-IIb study of MI, patients with diabetes mellitus had significantly higher 6-week (11.6% vs. 4.7%), 1-year (18.0% vs. 6.7%), and 3-year (21.6% vs. 9.6%) mortality rates compared to nondiabetic patients. The BARI trial, in which stents and abciximab were not used, showed that survival was better for patients with treated diabetes undergoing CABG with an arterial conduit than for those undergoing angioplasty. Stenting decreases the need for target revascularization procedures in diabetic patients compared with PTCA. The efficacy of stenting with GP IIb/IIIa inhibitors was assessed in the diabetic population compared to those without diabetes in a substudy of the EPISTENT trial. Irrespective of revascularization strategy abciximab significantly reduced 6-month death and MI rates in patients with diabetes for all strategies. Likewise, 6-month target-vessel revascularization was reduced in the stent/abciximab group approach.

7. Coronary Angioplasty After Coronary Artery Bypass Surgery

Although speculated to be at higher risk, patients having PCI of native vessels after prior coronary bypass surgery have, in recent years, nearly equivalent interventional outcomes and complication rates compared to patients having similar interventions without prior surgery. For PCI of SVG, studies indicate that the rate of successful angioplasty exceeds 90%, death <1.2%, Q-wave MI <2.5%. The incidence of non-Q-wave MI may be higher than that associated with native coronary arteries.

Use of GP IIb/IIIa blockers has not been shown to improve results of angioplasty in vein grafts. The native vessels should be treated with PCI if feasible. Patients with older and/or severely diseased SVGs may benefit from elective repeat coronary artery bypass graft surgery rather than PCI.

8. Specific Technical Considerations

Certain outcomes of PCI may be specifically related to the technology utilized for coronary recanalization. Antecedent unstable angina appears to be a clinical predictor of slow flow and periprocedural infarction following ablative technologies

TABLE 5. Clinical Risk Factors Associated With In-Hospital Adverse Events*

Variables	Definitions
Age	Date of birth as stated by the patient or family
Gender	Male or female
LVEF-calculated	Calculated by LV gram, echo, blood pool scan
LVEF-estimated	Estimated by LV gram, echo, blood pool scan
No. of vessels >70%	By angiography measured, quantified or estimated diameter stenosis; "vessel" defined as RCA and its branches, proximal LAD (before 1st diagonal), mid/distal LAD and its branches, and Cx and its branches
Unstable angina	Progressive or new onset or occurs at rest accompanied by ECG changes, hypotension or pulmonary congestion
CCS Class IV	Highest CCS angina class leading to hospital admission and/or intervention: 0 = no angina by Hx
CHF	Hx of CHF before intervention
MI at this admission	Within 24 h of AMI
Previous MI	>1 day; <7 days of AMI
Urgency of the procedure	<i>Elective:</i> patient clinically stable; procedure routinely scheduled <i>Urgent:</i> unstable patient; procedure scheduled before discharge <i>Emergent/ongoing ischemia:</i> ongoing ischemia including rest angina despite maximal therapy (medical or IABP) <i>Emergent/salvage:</i> arrest with CPR immediately before entering lab
Cardiogenic shock	Hypoperfusion with SBP <80 mm Hg and central filling pressure >20 mm Hg or cardiac index <1.8 liters/min/m ² ; also present if inotropes or IABP needed to maintain these values
Preprocedural IABP/CPS	IABP/CPS assisted device placed before intervention
Aortic valve disease	Aortic valve area <1.0 cm ² and/or Aortic regurgitation >2+
Mitral regurgitation >2+	Presence of mitral regurgitation >2+
Diabetes (treated)	Clinical diagnosis of diabetes treated either with oral agents or insulin with or without sequelae
PVD	Presence of occlusive disease in the aorta, iliac, or femoral artery sufficient to cause symptoms
Stroke	Hx of presence of fixed neurological deficit
Creatinine	If creatinine preintervention known, list creatinine
Creatinine >2 mg/dl	Creatinine >2 mg/dl known in past
Dialysis	Patient on dialysis
Cholesterol >225 mg/dl (reduced risk)	Measure cholesterol > 225 mg/dl before intervention
Same vessel intervention (reduced risk)	Any previous intervention on same vessel
Type C lesions attempted	Type A: concentric noncalcified, <10 mm in length, not bifurcated or angulated. Type C: total occlusion. Type B: all others (ACC/AHA)
LMCA attempted-unprotected	Intervention involving all or part of LMCA
LMCA attempted-protected	"Protected" LMCA stenosis by patent bypass conduit
Vein graft intervention	Any intervention to SVG or IMA
Thrombus	Intraluminal filling defect, haziness or contrast staining in artery before intervention

*Note: More than 50% of databases that evaluated the variable showed an odds ratio >2.0 or variable chosen on multivariable analysis. The definition of variables defined herein varies slightly from those agreed upon in the ACC Clinical Data Standards.

AMI = acute myocardial infarction; CCS = Canadian Cardiovascular Society; CHF = congestive heart failure; CPR = cardiopulmonary resuscitation; CPS = cardiopulmonary support; Cx = circumflex; ECG = electrocardiogram; Hx = history; IABP = intra-aortic balloon pump; IMA = internal mammary artery; LAD = left anterior descending coronary artery; LMCA = left main coronary artery; LV = left ventricle; LVEF = left ventricular ejection fraction; MI = myocardial infarction; PVD = peripheral vascular disease; RCA = right coronary artery; SBP = systolic blood pressure; SVG = saphenous vein graft. Adapted with permission from Block P, et al. J Am Coll Cardiol. 2000;32:275-82.

and direct platelet activation has been demonstrated to occur with both directional and rotational atherectomy.

Coronary perforation may occur more commonly following the use of ablative technologies including rotational, directional or extraction atherectomy, and excimer laser coronary angioplasty. Coronary perforation complicates PCI more frequently in the elderly and in women. While 20% of perforations may be secondary to the coronary guidewire, most are related to the specific technology used.

9. Issues of Hemodynamic Support in High-Risk Angioplasty

Elective high-risk PCI can be performed safely without intra-aortic balloon pump (IABP) or cardiopulmonary support (CPS) in most circumstances. Emergency high-risk PCI such as direct PCI for acute MI can usually be performed without IABP or CPS. CPS for high-risk PCI should be reserved only for patients at the extreme end of the spectrum

of hemodynamic compromise, such as those patients with extremely depressed LV function and patients in cardiogenic shock. However, it should be noted that in patients with borderline hemodynamics, ongoing ischemia, or cardiogenic shock, insertion of an intra-aortic balloon just prior to coronary instrumentation has been associated with improved outcomes. Furthermore, it is reasonable to obtain vascular access in the contralateral femoral artery prior to the procedure in patients in whom the risk of hemodynamic compromise is high, thereby facilitating intra-aortic balloon insertion, if necessary.

In patients having a higher-risk profile, consideration of alternative therapies, particularly CABG, formalized surgical standby, or periprocedural hemodynamic support should be addressed before proceeding with PCI.

F. Comparison With Bypass Surgery

The major advantage of PCI is its relative ease of use, avoiding general anesthesia, thoracotomy, extracorporeal circulation, CNS complications, and prolonged convalescence. Repeat PCI can be performed more easily than repeat bypass surgery, and revascularization can be achieved more quickly in emergency situations. The disadvantages of PCI are early restenosis and the inability to relieve many totally occluded arteries and/or those vessels with extensive atherosclerotic disease.

Coronary artery bypass surgery has the advantages of greater durability (graft patency rates exceeding 90% at 10 years with arterial conduits) and more complete revascularization irrespective of the morphology of the obstructing atherosclerotic lesion. Generally speaking, the greater the extent of coronary atherosclerosis and its diffuseness, the more compelling the choice of CABG, particularly if LV function is depressed. Patients with lesser extent of disease and localized lesions are good candidates for endovascular approaches.

Percutaneous transluminal coronary angioplasty and CABG have been compared in many nonrandomized and randomized studies. The most accurate comparisons of outcomes are best made from prospective randomized trials of patients suitable for either treatment. Although results of these trials provide useful information for selection of therapy in several patient subgroups, prior studies of PTCA may not reflect outcome of current PCI practice, which includes frequent use of stents and antiplatelet drugs. Similarly, many previous studies of CABG may not reflect outcome of current surgical practice in which arterial conduits are used whenever practicable. Beating heart bypass operations are also employed for selected patients with single-vessel disease with reduced morbidity. In addition, patients are selected for PCI (with or without stenting) because of certain lesion characteristics, and these anatomical criteria are not required for CABG.

Despite these limitations, some generalizations can be made from comparative trials of PTCA and CABG. First, for most patients with single-vessel disease, late survival is similar with either revascularization strategy, and this might be expected given the generally good prognosis of most patients with single-vessel disease managed medically.

In the ARTS trial, the first trial to compare stenting with surgery, there was no significant difference in mortality between PCI and surgical groups at one year. The main difference compared to previous PTCA and CABG trials was an approximate 50% reduction in the need for repeat revascularization in a group randomized to PCI with stent placement.

Direct comparison of initial strategies of PCI or CABG in patients with multivessel coronary disease is possible only by randomized trials because of selection criteria of patients for PCI. There have been five large (>300 patients) randomized trials of PTCA versus CABG and two smaller studies. These trials demonstrate that in appropriately selected patients with multivessel coronary disease, an initial strategy of standard PTCA yields similar overall outcomes (e.g., death, MI) compared to initial revascularization with coronary artery bypass.

An important exception to the conclusion of the relative safety of PCI in multivessel disease is the subgroup of patients with treated diabetes mellitus. Among treated diabetic patients in BARI assigned to PTCA, 5-year survival was 65.5% compared to 80.6% for patients having CABG ($p = 0.003$); the improved outcome with CABG was due to reduced cardiac mortality (5.8% vs. 20.6%, $p = 0.0003$), which was confined to those receiving at least one internal mammary artery graft.

G. Comparison With Medicine

There has been a considerable effort made to evaluate the relative effectiveness of bypass surgery as compared to PCI for coronary artery revascularization. In contrast to this, very little effort has been directed toward comparing medical therapy with PCI for the management of stable and unstable angina.

Based on the limited data available from randomized trials (Table 6) comparing medical therapy with PTCA, it seems prudent to consider medical therapy for the initial management of most patients with Canadian Cardiovascular Society Classification Class I and II and reserve PTCA and CABG for those patients with more severe symptoms and ischemia. The symptomatic individual patient who wishes to remain physically active, regardless of age, will more often require PCI. The results of the ACIP trial indicate that higher-risk patients with asymptomatic ischemia and significant CAD who undergo complete revascularization with CABG or PTCA may have a better outcome as compared to those with medical management.

IV. Institutional and Operator Competency

A. Quality Assurance

A mechanism for valid peer review must be established and ongoing at each institution performing PCI. Interventional cardiology procedures are associated with complications that in general are inversely related to operator and institutional volume. The mechanism for institutional review should provide an opportunity for interventionalists as well as physicians who do not perform angioplasty, but are knowledgeable about it, to review overall results of the program on

TABLE 6. PCI Comparison With Medical Therapy

Study	Year	N	Patient Population	Treatment	Follow-Up	Results		Significance	Comments
						PCI	Medical Therapy		
ACME	1992	212	Patients with single-vessel disease	Medical therapy vs. balloon angioplasty		64% less angina	46% less angina	$p < 0.01$	The PTCA group had less angina, better exercise performance and more improvement in quality of life scores, but had more complications (emergency bypass 2 patients, MI in 5, and repeat PTCA in 16).
VA ACME	1997	328	Patients with documented chronic stable angina 227 single-vessel disease 101 double-vessel disease	Medical therapy vs. balloon angioplasty	3 years	63% less angina	48% less angina	$p = 0.02$	Among patients with single-vessel disease, the PTCA group had less angina, better exercise performance, and more improvement in quality of life scores.
RITA-2	1997	1018	53% with Class II angina 47% with prior angina 7% triple-vessel disease	Medical therapy vs. balloon angioplasty	2.7 years	6.3% death or MI	3.3% death or MI	$p = 0.02$	The PTCA group had increased rates of death and MI, but had 7% less Class II angina at 2 years and longer exercise treadmill test time at 3 months.
ACIP	1997	558	Patients with documented CAD and asymptomatic ischemia 183 angina-guided drug therapy 183 angina plus ischemia-guided drug therapy 192 revascularization by PTCA or CABG	Angina-guided drug therapy vs. angina plus ischemia-guided drug therapy vs. revascularization	2 years	4.7% death or MI	8.8% death or MI for ischemia-guided drug therapy 12.1% death or MI for angina-guided drug therapy	$p < 0.01$	40% of patients had previous MI, 23% had prior PTCA or CABG and 38% had triple-vessel disease.
AVERT	1999	341	Patients with stable CAD, normal LV function and angina Class III	Medical therapy with atorvastatin vs PTCA	18 months	21% ischemic events	13% ischemic events	$p = 0.048$	$p = 0.045$ needed for significance due to interim analysis. Patients required to complete 4 minutes on Bruce protocol. Only 2 deaths among 341 patients in 18 months. Significant improvement in angina in patients treated with PTCA compared with medical therapy.

CABG = coronary artery bypass graft; CAD = coronary artery disease; LV = left ventricular; MI = myocardial infarction; PCI = percutaneous coronary intervention; PTCA = percutaneous transluminal coronary angioplasty.

TABLE 7. Key Components of a Quality Assurance Program

Clinical proficiency	<ul style="list-style-type: none"> • General indications/contraindications • Institutional and individual operator complication rates, mortality and emergency bypass surgery • Institutional and individual operator procedure volumes • Training and qualifications of support staff
Equipment maintenance and management	<ul style="list-style-type: none"> • Quality of laboratory facility (See ACC/SCAI Expert Consensus Document on Cardiac Catheterization Laboratory Standards)
Quality improvement process	<ul style="list-style-type: none"> • Establishment of an active concurrent database to track clinical and procedural information as well as patient outcomes for individual operators and the institution. The ACC-National Cardiovascular Data Registry™ is strongly recommended for this purpose
Radiation safety	<ul style="list-style-type: none"> • Educational program in the diagnostic use of X-ray • Patient and operator radiation exposure

a regular basis. The responsible supervising authority should monitor the following issues as outlined in Table 7.

The institutional credentialing committee should document that an interventionalist wishing to start practice meets the established training criteria, including those of the ACC Task Force on Training in Cardiac Catheterization and Interventional Cardiology. This Writing Committee agrees with the ACC Task Force recommendations for the Assessment and Maintenance of Proficiency in Coronary Interventional Procedures. Institutions performing PCI should meet the following standards as outlined in Tables 8 and 9.

B. Operator and Institutional Volume

The proliferation of small angioplasty or small surgical programs to support such angioplasty programs is strongly discouraged. Several studies have identified procedural volume as a determining factor for frequency of complications with PCI.

Although some investigators have suggested that low procedure volume does not contribute to poor outcomes, these studies are small in number and underpowered for analysis. Development of small cardiovascular surgical programs to support angioplasty is a poor use of resources that will likely lead to suboptimal results.

Given the concerns regarding operator volume and surgical standby, it is recommended that PCI be performed by higher volume operators (≥ 75 cases/year) with advanced technical skills (e.g., subspecialty certification) at institutions with fully equipped interventional laboratories and experienced support staff. This setting will most often be in a high-volume center (>400 cases/year) associated with an on-site cardiovascular surgical program. Similar concerns have been identified and

TABLE 8. Considerations for the Assessment and Maintenance of Proficiency in Coronary Interventional Procedures

Institutions
<ul style="list-style-type: none"> • Quality assessment monitoring of privileges and risk stratified outcomes • Provide support for a quality assurance staff person (e.g., nurse) to monitor complications • Minimal institutional performance activity of 200 interventions per year with the ideal minimum of 400 interventions per year • Interventional program director who has a career experience of >500 PCI procedures and is board certified by ABIM in interventional cardiology • Facility and equipment requirements to provide high resolution fluoroscopy and digital video processing • Experienced support staff to respond to emergencies (See Section IV, C. Need for Surgical Backup for discussion) • Establishment of a mentoring program for operators who perform <75 procedures per year by the individuals who perform ≥ 150 procedures per year
Physicians
<ul style="list-style-type: none"> • Procedural volume of ≥ 75 per year • Continuation of privileges based on outcome benchmark rates with consideration of not granting privileges to operators who exceed adjusted case mix benchmark complication rates for a 2-year-period • Ongoing quality assessment comparing results with current benchmarks with risk stratification of complication rates • Board Certification by ABIM in interventional cardiology

ABIM = American Board of Internal Medicine; PCI = percutaneous coronary intervention.

supported by the Task Force for Practice Guidelines for Coronary Angiography.

This Committee acknowledges that not every cardiologist desiring to do PCI should perform these procedures and not every hospital anxious to have an interventional program should start one. This caveat is particularly true where there are high-volume programs and operators nearby. In these situations, operators should be subspecialty board certified.

Recommendations for PCI Institutional and Operator Volumes at Centers With On-Site Cardiac Surgery

Class I

1. PCI done by operators with acceptable volume (≥ 75) at high-volume centers (>400). (*Level of Evidence: B*)

Class IIa

1. PCI done by operators with acceptable volume (≥ 75) at low-volume centers (200 to 400). (*Level of Evidence: C*)
2. PCI done by low volume operators (<75) at high-volume centers (>400). Note: Ideally operators with annual procedure volume <75 should only work at institutions with an activity level of >600 procedures/year.* (*Level of Evidence: C*)

Class III

1. PCI done by low volume operators (<75) at low-volume centers (200 to 400). Note: An institution with a volume <200 procedures/year, unless in a region that is underserved because of geography,

TABLE 9. Criteria for the Performance Angioplasty at Hospitals Without On-Site Cardiac Surgery

1. The operators must be experienced interventionalists who regularly perform elective intervention at a surgical center (≥ 75 cases/year). The institution must perform a minimum of 36 primary PCI procedures per year.
2. The nursing and technical catheterization laboratory staff must be experienced in handling acutely ill patients and comfortable with interventional equipment. They must have acquired experience in dedicated interventional laboratories at a surgical center. They participate in a 24-h, 365-day call schedule.
3. The catheterization laboratory itself must be well-equipped, with optimal imaging systems, resuscitative equipment, intra-aortic balloon pump (IABP) support, and must be well-stocked with a broad array of interventional equipment.
4. The cardiac care unit nurses must be adept in hemodynamic monitoring and IABP management.
5. The hospital administration must fully support the program and enable the fulfillment of the above institutional requirements.
6. There must be formalized written protocols in place for immediate (within 1 h) and efficient transfer of patients to the nearest cardiac surgical facility which are reviewed/tested on a regular (quarterly) basis.
7. Primary intervention must be performed routinely as the treatment of choice around the clock for a large proportion of patients with AMI, to ensure streamlined care paths and increased case volumes.
8. Case selection for the performance of primary angioplasty must be rigorous. Criteria for the types of lesions appropriate for primary angioplasty and for the selection for transfer for emergent aortocoronary bypass surgery are shown in Table 10.
9. There must be an ongoing program of outcomes analysis and formalized periodic case review.
10. Institutions should participate in a 3- to 6-month period of implementation during which time development of a formalized primary PCI program is instituted that includes establishing standards, training staff, detailed logistic development, and creation of a quality assessment and error management system.

Adapted with permission from Wharton TP Jr, McNamara NS, Fedele FA, Jacobs MI, Gladstone AR, Funk EJ. Primary angioplasty for the treatment of acute myocardial infarction: experience at two community hospitals without cardiac surgery. *J Am Coll Cardiol* 1999;33:1257-65.

AMI = acute myocardial infarction; IABP = intra-aortic balloon pump; PCI = percutaneous coronary intervention.

should carefully consider whether it should continue to offer service.* (Level of Evidence: C)

C. On-Site Cardiac Surgical Backup

Cardiac surgical backup for PCI has evolved from the formal surgical standby in the 1980s to an informal arrangement of first available operating room and, in some cases, off-site surgical backup. With the advent of intracoronary stenting, there has been a decrease in the need for emergency coronary bypass, ranging between 0.4 and 2%.

1. Primary PCI Without On-Site Cardiac Surgery

Although thrombolytic trials demonstrated that early reperfusion saves myocardium and reduces mortality, the superiority and greater applicability of primary PCI for the treat-

ment of acute MI has raised the question of whether primary PCI should be performed at institutions with diagnostic cardiac catheterization laboratories that do not perform elective PCI or have on-site cardiac surgery. For this reason, the establishment of PCI programs at institutions without on-site cardiovascular surgery has been promoted as necessary to maintain quality of care. It must be realized that PCI in the early phase of an acute MI can be difficult and requires even more skill and experience than routine PCI in the stable patient. The need for an experienced operator and experienced laboratory technical support with availability of a broad range of catheters, guidewires, stents, and other devices (e.g., IABP) that are required for optimum results in an acutely ill patient is of major importance (Table 9). If these complex patients are treated by interventionalists with limited experience at institutions with low volume, then the gains of early intervention may be lost because of increased complications. In such circumstances, transfer to a center that routinely performs complex PCI will often be a more effective and efficient course of action. Thrombolysis is still an acceptable form of therapy and is preferable to acute PCI by an inexperienced team.

Criteria have been suggested for the performance of primary PCI at hospitals without on-site cardiac surgery (Tables 9 and 10). Of note, large-scale registries have shown an inverse relationship between the number of primary angioplasty procedures performed and in-hospital mortality. The data suggest that both door-to-balloon time and in-hospital mortality are significantly lower in institutions performing a minimum of 36 primary angioplasty procedures per year. Communities may identify a unique qualified and experienced center wherein the on-site intervention for acute MI could be performed. Suboptimal results may relate to operator/staff inexperience and capabilities and delays in performing angioplasty for logistical reasons. From clinical data and expert consensus, the Committee recommends that primary PCI for acute MI performed at hospitals without established elective PCI programs should be restricted to those institutions with a proven plan for rapid and effective PCI as well as rapid access to cardiac surgery in a nearby facility (Table 11).

2. Elective PCI Without On-Site Surgery

Technical improvements in interventional cardiology have led to the development of elective angioplasty programs without on-site surgical coverage. Caution is warranted before endorsing an unrestricted policy for PCI in hospitals without appropriate facilities. Several outstanding and critically important clinical issues, such as timely management of ischemic complications, adequacy of specialized post-interventional care, logistics for managing cardiac surgical or vascular complications and operator/laboratory volumes, and accreditation must be addressed. At this time, the Committee, therefore, continues to support the recommendation that elective PCI should not be performed in facilities without on-site cardiac surgery (Table 11). As with many dynamic areas in interventional cardiology, these recommendations may be subject to revision as clinical data and experience increase.

*Operators who perform <75 procedures/year should develop a defined mentoring relationship with a highly experienced operator who has an annual procedural volume >150 procedures/year.

TABLE 10. Patient Selection for Angioplasty and Emergency Aortocoronary Bypass at Hospitals Without On-Site Cardiac Surgery

Avoid intervention in hemodynamically stable patients with:

- Significant ($\geq 60\%$) stenosis of an unprotected left main (LM) coronary artery upstream from an acute occlusion in the left coronary system that might be disrupted by the angioplasty catheter
- Extremely long or angulated infarct-related lesions with TIMI grade 3 flow
- Infarct-related lesions with TIMI grade 3 flow in stable patients with 3-vessel disease
- Infarct-related lesions of small or secondary vessels
- Lesions in other than the infarct artery

Transfer for emergent aortocoronary bypass surgery patients with:

- High-grade residual left main or multivessel coronary disease and clinical or hemodynamic instability
 - After angioplasty or occluded vessels
 - Preferably with intraaortic balloon pump support

Adapted with permission from Wharton TP Jr, McNamara NS, Fedele FA, Jacobs MJ, Gladstone AR, Funk EJ. Primary angioplasty for the treatment of acute myocardial infarction: experience at two community hospitals without cardiac surgery. *J Am Coll Cardiol* 1999;33:1257–65.

Recommendations for PCI With and Without On-Site Cardiac Surgery (Table 11)

Class I

1. Patients undergoing elective PCI in facilities with on-site cardiac surgery. (*Level of Evidence: B*)
2. Patients undergoing primary PCI in facilities with on-site cardiac surgery. (*Level of Evidence: B*)

Class IIb

1. Patients undergoing primary PCI in facilities without on-site cardiac surgery, but with a proven plan

for rapid access (within 1 h) to a cardiac surgery operating room in a nearby facility with appropriate hemodynamic support capability for transfer. The procedure should be limited to patients with ST-segment elevation MI or new LBBB on ECG, and done in a timely fashion (balloon inflation within 90 ± 30 min of admission) by persons skilled in the procedure (≥ 75 PCIs/year) and only at facilities performing a minimum of 36 primary PCI procedures per year. (*Level of Evidence: B*)

Class III

1. Patients undergoing elective PCI in facilities without on-site cardiac surgery. (*Level of Evidence: C*)
2. Patients undergoing primary PCI in facilities without on-site cardiac surgery and without a proven plan for rapid access (within 1 h) to a cardiac surgery operating room in a nearby facility with appropriate hemodynamic support capability for transfer or when performed by lower skilled operators (< 75 PCIs/year) in a facility performing < 36 primary PCI procedures per year. (*Level of Evidence: C*)

V. Indications

A broad spectrum of clinical presentations exists wherein patients may be considered candidates for PCI, ranging from asymptomatic to severely symptomatic or unstable, with variable degrees of jeopardized myocardium. Each time that a patient is considered for revascularization, the potential risk and benefits of the particular procedure under consideration must be weighed against alternative therapies.

The initial simplicity and associated low morbidity of PCI as compared to surgical therapy is always attractive, but the patient and family must understand the limitations inherent in

TABLE 11. Recommendations For PCI With and Without On-Site Cardiac Surgery

With On-Site Cardiac Surgery		Without On-Site Cardiac Surgery
Elective PCI	Class I Patients undergoing elective PCI in facilities with on-site cardiac surgery. (<i>Level of Evidence: B</i>)	Class III Patients undergoing elective PCI in facilities without on-site cardiac surgery. (<i>Level of Evidence: C</i>)
	Primary PCI Class I Patients undergoing primary PCI in facilities with on-site cardiac surgery. (<i>Level of Evidence: B</i>)	Class IIb Patients undergoing primary PCI in facilities without on-site cardiac surgery, but with a proven plan for rapid access (within 1 h) to a cardiac surgery operating room in a nearby facility with appropriate hemodynamic support capability for transfer. The procedure should be limited to patients with ST-segment elevation MI or new LBBB on ECG, and done in a timely fashion (balloon inflation within 90 ± 30 min of admission) by persons skilled in the procedure (≥ 75 PCIs/year) and only at facilities performing a minimum of 36 primary PCI procedures per year. (<i>Level of Evidence: B</i>)
		Class III Patients undergoing primary PCI in facilities without on-site cardiac surgery and without a proven plan for rapid access (within 1 h) to a cardiac surgery operating room in a nearby facility with appropriate hemodynamic support capability for transfer. (<i>Level of Evidence: C</i>)

ECG = electrocardiography; LBBB = left bundle-branch block; MI = myocardial infarction; PCI = percutaneous coronary intervention.

current PCI procedures, including a realistic presentation of the likelihood of restenosis and the potential for incomplete revascularization as compared with CABG surgery. In patients with CAD who are asymptomatic or have only mild symptoms, the potential benefit of antianginal drug therapy along with an aggressive program of risk reduction must also be understood by the patient before a revascularization procedure is performed.

A. Asymptomatic or Mild Angina

In the previous ACC/AHA Guidelines for PTCA, specific recommendations were made separately for patients with single- or multivessel disease. The current techniques of PCI have matured to the point where, in patients with favorable anatomy, the competent practitioner can perform either single- or multivessel PCI at low risk and with a high likelihood of initial success. For this reason, in this revision of the Guidelines, recommendations will be made largely based upon the patients' clinical condition, specific coronary lesion morphology and anatomy, LV function, and associated medical conditions, and less emphasis will be placed on the number of lesions or vessels requiring PCI. The CCS Class of angina (I to IV) is used to define the severity of symptoms. The categories described in this section refer to an initial PCI procedure in a patient without prior CABG surgery.

The Committee recognizes that the majority of patients with asymptomatic ischemia or mild angina should be treated medically. The published ACIP study casts some doubt on the wisdom of medical management for those higher-risk patients who are asymptomatic or have mild angina, but have objective evidence by both treadmill testing and ambulatory monitoring of significant myocardial ischemia and CAD. In addition, there is a substantial portion of the middle and older age populations in this country that remains physically active, participating in sports, such as tennis and skiing, or performing regular and vigorous physical exercise, such as jogging, who have CAD. For such individuals with moderate or severe ischemia and few symptoms, revascularization with PCI or CABG surgery may reduce their risk of serious or fatal cardiac events. For this reason, patients in this category of higher-risk asymptomatic ischemia or mild symptoms and severe anatomic CAD are placed in Class I or II. PCI may be considered if there is a high likelihood of success and a low risk of morbidity or mortality. The judgment of the experienced physician is deemed valuable in assessing the extent of ischemia.

Recommendations for PCI in Asymptomatic or Class I Angina Patients

Class I

1. Patients who do not have treated diabetes with asymptomatic ischemia or mild angina with 1 or more significant lesions in 1 or 2 coronary arteries suitable for PCI with a high likelihood of success and a low risk of morbidity and mortality. The vessels to be dilated must subtend a large area of viable myocardium (Table 12). (*Level of Evidence: B*)

TABLE 12. Noninvasive Risk Stratification: High Risk (>3% Annual Mortality Rate)

- High-risk treadmill score (score ≤ -11)
- Stress-induced large perfusion defect (particularly if anterior)
- Stress-induced perfusion defects of moderate size
- Stress-induced multiple perfusion defect with LV dilation or increased lung uptake (thallium-201)
- Echocardiographic wall motion abnormality (involving >2 segments) developing at a low dose of dobutamine ($\leq 10 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) or at a low heart rate (120 bpm)
- Stress echocardiographic evidence of extensive ischemia

Adapted with permission from Gibbons RJ, Chatterjee K, Daley J, et al. ACC/AHA/ACP-ASIM guidelines for the management of patients with chronic stable angina. J Am Coll Cardiol 1999;33:2092-197.

Class IIa

1. The same clinical and anatomic requirements for Class I, except the myocardial area at risk is of moderate size or the patient has treated diabetes. (*Level of Evidence: B*)

Class IIb

1. Patients with asymptomatic ischemia or mild angina with ≥ 3 coronary arteries suitable for PCI with a high likelihood of success and a low risk of morbidity and mortality. The vessels to be dilated must subtend at least a moderate area of viable myocardium. In the physician's judgment, there should be evidence of myocardial ischemia by ECG exercise testing, stress nuclear imaging, stress echocardiography or ambulatory ECG monitoring, or intracoronary physiologic measurements. (*Level of Evidence: B*)

Class III

1. Patients with asymptomatic ischemia or mild angina who do not meet the criteria as listed under Class I or Class II and who have:
 - a. Only a small area of viable myocardium at risk.
 - b. No objective evidence of ischemia.
 - c. Lesions that have a low likelihood of successful dilation.
 - d. Mild symptoms that are unlikely to be due to myocardial ischemia.
 - e. Factors associated with increased risk of morbidity or mortality.
 - f. Left main disease.
 - g. Insignificant disease <50%. (*Level of Evidence: C*)

B. Angina Class II to IV or Unstable Angina

Many patients with moderate or severe stable angina or unstable angina do not respond adequately to medical therapy and often have significant coronary artery stenoses that are suitable for revascularization with CABG surgery or PCI. In addition, a proportion of these patients have reduced LV systolic function which places them in a group that is known to have improved survival with CABG surgery and possibly

with revascularization by PCI. In nondiabetic patients with 1- or 2-vessel disease in whom angioplasty of 1 or more lesions has a high likelihood of initial success, PCI is the preferred approach. In a minority of such patients, CABG surgery may be preferred, particularly for those in whom the left anterior descending coronary artery can be revascularized with the internal mammary artery or in those with left main coronary disease. In patients with unstable angina or non-Q-wave MI, intensive medical therapy should be initiated prior to revascularization with PCI or CABG surgery. Patients with unstable angina and non-ST-segment elevation MI have been randomized to medical therapy or PCI in the FRISC II and TACTICS TIMI 18 trials. These trials utilizing stenting as the primary therapy have favored the invasive approach.

The indications for coronary angiography are summarized in the ACC/AHA Coronary Angiography Guidelines and recommendations for PCI are summarized in the ACC/AHA Unstable Angina Guidelines. Indications for PCI for patients with angina Class II to IV, unstable angina, or non-Q-wave infarction follow.

Recommendations for Patients With Moderate or Severe Symptoms (Angina Class II to IV, Unstable Angina or Non-ST-Elevation MI) With Single- or Multivessel Coronary Disease on Medical Therapy

Class I

1. Patients with 1 or more significant lesions in 1 or more coronary arteries suitable for PCI with a high likelihood of success and low risk of morbidity or mortality (Table 5). The vessel(s) to be dilated must subtend a moderate or large area of viable myocardium and have high risk (Table 12). (*Level of Evidence: B*)

Class IIa

1. Patients with focal saphenous vein graft lesions or multiple stenoses who are poor candidates for reoperative surgery. (*Level of Evidence: C*)

Class IIb

1. Patient has 1 or more lesions to be dilated with reduced likelihood of success (Table 5) or the vessel(s) subtend a less than moderate area of viable myocardium. Patients with 2- or 3-vessel disease, with significant proximal LAD CAD and treated diabetes or abnormal LV function. (*Level of Evidence: B*)

Class III

1. Patient has no evidence of myocardial injury or ischemia on objective testing and has not had a trial of medical therapy, or has:
 - a. Only a small area of myocardium at risk.
 - b. All lesions or the culprit lesion to be dilated with morphology with a low likelihood of success.
 - c. A high risk of procedure-related morbidity or mortality. (*Level of Evidence: C*)

TABLE 13. Invasive vs Conservative Strategies in Unstable Angina Patients

Study	Year	N	Patient Population	Treatment	Follow-Up	Results			Comments
						PCI	Medical Therapy	Significance	
TIMI-IIIb	1995	1473	Patients 21–76 years of age presenting within 24 h of ischemic discomfort at rest consistent with unstable angina or non-Q-wave MI	Medical therapy (TPA vs. placebo) and early invasive or conservative strategy	6 weeks	16.2% combined primary endpoints	18.1% combined primary endpoints	NS	While no difference was found in combined primary endpoints (death, MI, positive ETT), the early invasive strategy was associated with shorter hospital stay and lower incidence of rehospitalization
VANQWISH	1998	920	Patients with an evolving MI	Invasive vs conservative	1 year Avg 23 months	12.4% 32.9% death and MI	10.6% 30.3% death and MI	NS p = 0.35	Fewer patients treated conservatively had death plus MI or death at hospital discharge at 1 month and at 1 year. The invasive group had a higher CABG mortality rate (11.6% vs. 3.4%)
FRISC II	1999	2,457	Patient's ischemic symptoms in previous 48 h accompanied by ECG changes or elevated markers	Early invasive therapy or noninvasive treatment strategy. Patients also received diltiazem or placebo for 3 months	6 months	9.4% death or MI	12.1% death or MI	p = 0.031	Invasive strategy was associated with 50% lower recurrent angina and hospital readmission rates

CABG = coronary artery bypass graft; ECG = electrocardiography; ETT = exercise treadmill test; MI = myocardial infarction; NS = no significance; PCI = percutaneous coronary intervention.

2. Patients with insignificant coronary stenosis (e.g., <50% diameter). (Level of Evidence: C)
3. Patients with significant left main CAD who are candidates for CABG. (Level of Evidence: B)

It is recognized by the Committee that the assessment of risk of unsuccessful PCI or serious morbidity or mortality must always be made with consideration of the alternative therapies available for the patient, including more intensive or prolonged medical therapy or surgical revascularization (Table 13), especially in patients with unstable angina pectoris.

When CABG surgery is a poor option because of high risk due to special considerations or other organ system disease, patients otherwise in Class IIb may be appropriately managed with PCI. Under these special circumstances formal surgical consultation is recommended.

C. Myocardial Infarction

The results of randomized clinical trials of intravenous thrombolysis and subsequent management strategies of immediate, delayed, and deferred PCI have established the benefits of early pharmacologic and mechanical reperfusion therapies for patients with acute MI.

Percutaneous coronary intervention is a very effective method for re-establishing coronary perfusion and is suitable for $\geq 90\%$ of patients. Considerable data support the use of PCI for patients with acute MI. Reported rates of achieving TIMI 3 flow, the goal of reperfusion therapy, range from 70 to 90%. Late follow-up angiography demonstrates that 87% of infarct arteries remain patent. Although most evaluations of PCI have been in patients who are eligible to receive thrombolytic therapy, considerable experience supports the value of PCI for patients who may not be suitable for thrombolytic therapy due to an increased risk of bleeding.

Intracoronary stents appear to augment the results of PCI for MI (Table 14). Preliminary results suggest that stenting achieves a better immediate angiographic result with a larger arterial lumen, less reclosure of the infarct-related artery, and fewer subsequent ischemic events than PTCA alone. Results from a randomized clinical trial suggest that stenting enhances late clinical outcomes (reduction in composite end point attributable to a decrease in target-vessel revascularization) when compared to PTCA alone. However an increase in mortality at 1 year among the stent group has been reported in the Stent-PAMI trial.

Primary PTCA performed without routine stenting has been compared to thrombolytic therapy in several randomized clinical trials. These investigations consistently demonstrate that PTCA-treated patients experience less recurrent ischemia or infarction than those treated by thrombolysis. Trends favoring a survival benefit with PTCA are noted. Two meta-analyses showed superiority of PCI over thrombolysis for mortality with risk reductions of 0.34 and 0.56. It is important to note that these results of PCI have been achieved in medical centers with experienced providers and under circumstances where angioplasty can be performed immediately following patient presentation.

1. PCI in Thrombolytic-Ineligible Patients

Randomized, controlled clinical trials evaluating the outcome of PCI for patients who present with ST-segment elevation

TABLE 14. Studies Comparing PTCA With Stents in Acute Myocardial Infarction

Study	Year	Follow-Up Month	N, Stent/ Angioplasty	Success %	Crossover %	Early Events (0–30 days), %				Late Events (Cumulative), %				
						Death	Reinfarction	TVR	Any Event	Restenosis	Death	Reinfarction	TVR	Any Event
GRAMI	1998	12	52/52	98/94.2	25*	3.8/7.6	0/7.6	0/5.7	3.8/19.2	—	—	—	14/21	17/35
FRESCO	1998	6	75/75	99†	—	0/0	1.3/2.6	1.3/12	3/15	17/43	1/0	1/3	7/25	13/32
STENTIM 2	2000	12	101/110	95/94.5	3/36.4	1/0	4/3.6	5/5.4	5/5.4	25.3/39.6	3/1.9	4.0/5.5	17.8/28.2	12.9/20.0
Suryapranata et al.	1998	6	112/115	98/96	2/13	2/3	1/4	—	—	—	2/3	1/7	4/17	5/20
PASTA	1999	12	67/69	99/97	1/10	3/7	3/4	6/13	6/19	17/37.5	5/9	—	—	22/49
SAAMI	2001	710 ± 282 days	44/44	—	1/27	2/5	0/2	0/9	5/11	24/61	9/18	2/9	16/34	23/43
Stent-PAMI	1999	6	452/448	89.4/92.7	1.5/15	3.5/1.8	0.4/1.1	1.8/3.8	4.6/5.8	20.3/33.5	4.2/2.7	2.4/2.2	7.7/17	12.6/20.1

*Values for crossovers from angioplasty to stent treatment; †Success rate of 99% before randomization

Dashes — = data not gathered for that category; TVR = target-vessel revascularization. All data are presented as values for stent/angioplasty groups. Adapted from Suwaidi MB, et al. JAMA 2000;284:1828–1836.

TABLE 15. Contraindications and Cautions for Thrombolytic Use in Myocardial Infarction***Contraindications**

- Previous hemorrhagic stroke at any time, other strokes or cerebrovascular events within 1 year
- Known intracranial neoplasm
- Active internal bleeding (does not include menses)
- Suspected aortic dissection

Caution/relative contraindications

- Severe uncontrolled hypertension on presentation (blood pressure >180/110 mm Hg)†
- History of prior cerebrovascular accident or known intracerebral pathology not covered in contraindications
- Current use of anticoagulants in therapeutic doses (INR $\geq 2-3$); known bleeding diathesis
- Recent trauma (within 2–4 weeks) including head trauma or traumatic or prolonged (>10 min) CPR or major surgery (3 weeks)
- Noncompressible vascular punctures
- Recent (within 2 to 4 weeks) internal bleeding
- For streptokinase/anistreplase: prior exposure (especially within 5 days–2 years) or prior allergic reaction
- Pregnancy
- Active peptic ulcer
- History of chronic severe hypertension

*Viewed as advisory for clinical decision making and may not be all-inclusive or definitive; †Could be an absolute contraindication in low-risk patients with myocardial infarction.

INR = International Normalized Ratio; CPR = cardiopulmonary resuscitation.

Reproduced with permission from Ryan TJ, Antman EM, Brooks NH, et al. ACC/AHA guidelines for the management of patients with acute myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Management of Acute Myocardial Infarction). *J Am Coll Cardiol* 1999;34:890–911.

but who are ineligible for thrombolytic therapy and for patients who experience infarction without ST-segment elevation have not been performed. Nevertheless, there is a general consensus that PCI is an appropriate means for achieving reperfusion in patients who cannot receive thrombolytics because of increased risk of hemorrhage. Other reasons also exclude acute MI patients from thrombolytic therapy and the outcome of PCI in these patients may differ from those eligible for lytic therapy. For example, patients who present without ST-elevation are more often older and female and have higher in-hospital mortality than those with ST-segment elevation. Little data are available to characterize the value of primary PCI for this subset of acute MI patients (Table 15).

2. Post-Thrombolysis PCI

In asymptomatic patients, the strategies of routine PCI of the stenotic infarct-related artery immediately after successful thrombolysis show no benefit with regard to salvage of jeopardized myocardium or prevention of reinfarction or death. In some studies this approach was associated with increased incidence of adverse events, which include bleeding, recurrent ischemia, emergency coronary artery surgery, and death. Routine PCI immediately after thrombolysis may increase the chance for vascular complications at the catheterization access site and hemorrhage into the infarct-related vessel wall.

terization access site and hemorrhage into the infarct-related vessel wall.

3. Rescue PCI

Rescue (also known as salvage) PCI is defined as PCI after failed thrombolysis for patients with continuing or recurrent myocardial ischemia. Rescue PCI has resulted in higher rates of early infarct-artery patency, improved regional infarct zone wall motion, and greater freedom from adverse in-hospital clinical events compared to a deferred PCI strategy. The randomized evaluation of rescue PCI with combined utilization end points trial (RESCUE) demonstrated a reduction in rates of in-hospital death and combined death and congestive heart failure maintained up to 1 year after study entry for patients presenting with anterior wall MI who failed thrombolytic therapy. Improvement in TIMI grade flow from ≤ 2 to 3 may offer additional clinical benefit.

4. PCI for Cardiogenic Shock

Observational studies support the value of PCI for patients who develop cardiogenic shock in the early hours of MI. For patients who do not have mechanical causes of shock, such as acute mitral regurgitation or septal or free wall rupture, mortality among those having PCI is lower than those treated by medical means.

A randomized clinical trial has further clarified the role of emergency revascularization (ERV) in acute MI complicated by cardiogenic shock. This multicenter trial supports the use of ERV with PCI in appropriate candidates for patients <75 years old with acute MI complicated by cardiogenic shock. After 6 months, there was significant survival benefit to early revascularization. These data strongly support the approach that patients <75 years with acute MI complicated by cardiogenic shock should undergo emergency revascularization and support measures.

5. PCI Hours to Days After Thrombolysis

Patients who achieve reperfusion and myocardial salvage following thrombolytic therapy may experience reocclusion of the infarct artery and recurrent MI. This concern has prompted the routine use of catheterization and PCI prior to hospital discharge to identify and dilate the culprit lesion. The SWIFT study examined 800 patients with acute MI randomly assigned to PCI within 2 to 7 days after thrombolysis or to conservative management with intervention for spontaneous or provokable ischemia. There were no differences in the two treatment strategies regarding LV function, incidence of reinfarction, in-hospital survival, or 1-year survival rate. These data indicate that routine PCI of the infarct-related artery in the absence of spontaneous or provoked ischemia is not warranted.

Initial studies of late (>6 to 12 h) PCI in asymptomatic survivors of MI indicate that opening an occluded artery does not appear to alter the process of LV dilation, the incidence of spontaneous and inducible arrhythmias, or prognosis. Although data supporting the argument to open occluded infarct-related arteries are persuasive, at least for large arteries subtending large areas of myocardium, there are few randomized trials supporting this approach. It should be noted that the overwhelming majority of trials were performed prior

to the widespread use of stents and platelet IIb/IIIa receptor blockade and thus, the potential impact and benefit of these newer therapies in this clinical setting needs re-evaluation.

6. PCI After Thrombolysis in Selected Patient Subgroups

a. Young and Elderly Post-Infarct Patients

Although not supported by randomized trials, routine cardiac catheterization following thrombolytic therapy for AMI has been a frequently performed strategy in all age groups. Young (<50 years) patients often undergo cardiac catheterization after thrombolytic therapy due to a "perceived need" to define coronary anatomy and thus establish psychological as well as clinical outcomes. In contrast, older (>75 years) patients have higher in-hospital and long-term mortality rates and enhanced clinical outcomes when treated with primary PCI. Confirmatory studies to determine quality-of-life aspects of care in younger patients and to define the potential of other modes of coronary revascularization in older patient groups are not yet available. Based on the current data, with the exception of patients presenting with cardiogenic shock, PCI should be based on clinical need without special consideration of age.

b. Patients With Prior Myocardial Infarction

A prior MI is an independent predictor of death, reinfarction, and need for urgent coronary bypass surgery. In the TIMI-II study, patients with a history of prior MI had a higher 42-day mortality (8.8% vs. 4.3%; $p < 0.001$), higher prevalence of multivessel CAD (60% vs. 28%; $p < 0.001$), and a lower LV ejection fraction (42% vs. 48%; $p < 0.001$) compared to patients with a first MI. Mortality tended to be lower among patients with a prior MI undergoing the invasive compared to the conservative strategy, a benefit which persisted up to 1 year following study entry.

Based on the earlier findings in this document and current practice, PCI should be based on clinical need. The presence of prior MI places the patient in a higher risk subset and should be considered in the PCI decision.

Recommendations for Primary PCI for Acute Transmural MI Patients as an Alternative to Thrombolysis

Class I

1. As an alternative to thrombolytic therapy in patients with AMI and ST-segment elevation or new or presumed new left bundle branch block who can undergo angioplasty of the infarct artery ≤ 12 h from the onset of ischemic symptoms or >12 h if symptoms persist, if performed in a timely fashion* by individuals skilled in the procedure† and supported by experienced personnel in an appropriate laboratory environment‡ (Level of Evidence: A)
2. In patients who are within 36 h of an acute ST elevation/Q-wave or new left bundle branch block MI who develop cardiogenic shock, are <75 years of age, and revascularization can be performed within 18 h of the onset of shock by individuals skilled in the procedure† and supported by experienced personnel

in an appropriate laboratory environment‡ (Level of Evidence: A)

Class IIa

1. As a reperfusion strategy in candidates who have a contraindication to thrombolytic therapy. (Level of Evidence: C)

Class III

1. Elective PCI of a non-infarct-related artery at the time of acute MI. (Level of Evidence: C)
2. In patients with acute MI who:
 - a. have received fibrinolytic therapy within 12 h and have no symptoms of myocardial ischemia.
 - b. are eligible for thrombolytic therapy and are undergoing primary angioplasty by an inexperienced operator (individual who performs <75 PCI procedures/year).
 - c. are beyond 12 h after onset of symptoms and have no evidence of myocardial ischemia. (Level of Evidence: C)

Recommendations for PCI After Thrombolysis

Class I

1. Objective evidence for recurrent infarction or ischemia (rescue PCI). (Level of Evidence: B)

Class IIa

1. Cardiogenic shock or hemodynamic instability. (Level of Evidence: B)

Class IIb

1. Recurrent angina without objective evidence of ischemia/infarction. (Level of Evidence: C)
2. Angioplasty of the infarct-related artery stenosis within hours to days (48 h) following successful thrombolytic therapy in asymptomatic patients without clinical and/or inducible evidence of ischemia. (Level of Evidence: B)

Class III

1. Routine PCI within 48 h following failed thrombolysis. (Level of Evidence: B)
 2. Routine PCI of the infarct-artery stenosis immediately after thrombolytic therapy. (Level of Evidence: A)
- Recommendations for PCI During Subsequent Hospital Management After Acute Therapy for AMI Including Primary PCI*

Class I

1. Spontaneous or provokable myocardial ischemia during recovery from infarction. (Level of Evidence: C)
2. Persistent hemodynamic instability. (Level of Evidence: C)

Class IIa

1. Patients with LV ejection fraction ≤ 0.4 , CHF, or serious ventricular arrhythmias. (Level of Evidence: C)

*Performance standard: balloon inflation within 90 (± 30) min of hospital admission; †Individuals who perform ≥ 75 PCI procedures/year; ‡Centers that perform >200 PCI procedures/year and have cardiac surgical capability.

Class IIb

1. Coronary angiography and angioplasty for an occluded infarct-related artery in an otherwise stable patient to revascularize that artery (open artery hypothesis). (*Level of Evidence: C*)
2. All patients after a non-Q-wave MI. (*Level of Evidence: C*)
3. Clinical HF during the acute episode, but subsequent demonstration of preserved LV function (LV ejection fraction >0.4). (*Level of Evidence: C*)

Class III

1. PCI of the infarct-related artery within 48 to 72 h after thrombolytic therapy without evidence of spontaneous or provokable ischemia. (*Level of Evidence: C*)

D. Percutaneous Intervention in Patients With Prior Coronary Bypass Surgery

Ischemic symptoms recur in 4% to 8% of patients/year following CABG. Recurrence of symptoms can be attributed to progression of native vessel coronary disease (5%/year) and bypass conduit occlusion, particularly SVG failure (7% in week 1; 15 to 20% in first year; 1 to 2%/year during the first 5 to 6 years and 3 to 5%/year in years 6 to 10 postoperatively). At 10 years postoperatively, approximately half of all SVG conduits are occluded and only half of the remaining patent grafts are free of significant disease. The requirement for repeat revascularization procedures increases over time from the initial revascularization, particularly in younger patients. Although arterial conduits exhibit improved long-term patency, stenosis or occlusion of these grafts can occur. Thus, patients with recurrent ischemic symptoms following CABG may require repeat revascularization due to diverse anatomic problems.

Risk of repeat surgical revascularization is higher (hospital mortality 7 to 10%) than initial CABG and both long-term relief of angina and bypass graft patency are lower than that of the first procedure. In addition, patients with prior bypass surgery may have limited graft conduits, impaired LV function, advanced age, and coexisting medical conditions (cerebrovascular disease; renal and pulmonary insufficiency) which may complicate repeat surgical coronary revascularization and prompt consideration for catheter-based intervention.

1. Early Ischemia After CABG

Recurrent ischemia early (<30 days) postoperatively usually reflects graft failure, often secondary to thrombosis, and may occur in both saphenous vein and arterial graft conduits. Incomplete revascularization and unbypassed native vessel stenoses or stenoses distal to a bypass graft anastomosis may also precipitate recurrent ischemia. Urgent coronary angiography is indicated to define the anatomic cause of ischemia and to determine the best course of therapy. Emergency PCI of a focal graft stenosis (venous or arterial) or recanalization of an acute graft thrombosis may successfully relieve ischemia in the majority of patients. Balloon dilation across suture lines has been accomplished safely within days of surgery. Adjunctive therapy with abciximab for percutaneous

intervention during the first week following bypass surgery has been limited but intuitively may pose less risk for hemorrhage than fibrinolysis. As flow in vein graft conduits is pressure dependent, intra-aortic balloon pump support should be considered in the context of systemic hypotension and/or severe LV dysfunction. If feasible, PCI of both bypass graft and native vessel offending stenoses should be attempted, particularly if intracoronary stents can be successfully deployed.

When ischemia occurs 1 to 12 months following surgery, the etiology is usually peri-anastomotic graft stenosis. Distal anastomotic stenoses (both arterial and venous) respond well to balloon dilation alone and have a more favorable long-term prognosis than stenoses involving the mid-shaft or proximal vein graft anastomosis. The immediate results of PCI in mid-shaft ostial or distal anastomotic vein graft stenoses may be enhanced by coronary stent deployment.

Percutaneous transluminal coronary angioplasty with or without stent deployment can be successfully performed in patients with distal anastomotic stenoses involving the gastroepiploic artery bypass graft and in patients with free radial artery bypass grafts as well. Percutaneous intervention has also been effective in relieving ischemia for patients with the stenosis of the subclavian artery proximal to the origin of a patent left internal mammary artery bypass graft.

2. Late Ischemia After CABG

Ischemia occurring more than 1 year postoperatively usually reflects the development of new stenoses in graft conduits and/or native vessels that may be amenable to PCI. Slow-flow occurs more frequently in grafts having diffuse atherosclerotic involvement, angiographically demonstrable thrombus, irregular or ulcerative lesion surfaces, and with long lesions having large plaque volume.

Final patency after PTCA is greater for distal SVG lesions than for ostial or mid-SVG lesions, and stenosis location appears to be a better determinant of final patency than graft age or the type of interventional device used.

Percutaneous intervention for chronic vein graft occlusion has been problematic. Percutaneous transluminal coronary angioplasty alone has been associated with high complication rates and low rates of sustained patency. Favorable results have been obtained with both local "targeted" and more prolonged infusion of fibrinolytic agents for nonocclusive intragraft thrombus. Thrombolytic catheter-based systems appear to successfully treat SVG thrombosis as well as or better than thrombolytic agents.

3. Early and Late Outcomes of Percutaneous Intervention

Patients with prior bypass surgery who undergo successful PCI have a long-term outcome that is dependent on patient age, the degree of LV dysfunction, and the presence of multivessel coronary atherosclerosis. The best long-term results are observed after recanalization of distal anastomotic stenoses occurring within 1 year of operation. Conversely, event-free survival is less favorable following angioplasty of totally occluded SVGs, ostial vein graft stenoses, or grafts with diffuse or multicentric disease. Coexistent multisystemic disease, the presence of which may have prompted the choice

of a percutaneous revascularization strategy, may also influence long-term outcomes in this population.

4. Surgery Versus Percutaneous Reintervention

Aged, diffuse, friable and degenerative SVG disease in the absence of a patent arterial conduit to the left anterior descending artery represents a prime consideration for repeat surgical revascularization. The overall risk of repeat operation, especially the presence of comorbidities such as concomitant cerebrovascular, renal, or pulmonary disease and the potential for jeopardizing patent, nondiseased bypass conduits must be carefully considered. Isolated, friable stenoses in vein grafts may be approached with primary stenting or the combination of extraction atherectomy and stenting in an attempt to reduce the likelihood of distal embolization.

In general, patients with multivessel disease, failure of multiple SVGs, and moderately impaired LV function, derive the greatest benefit from the durability provided by surgical revascularization with arterial conduits. Regardless of repeat revascularization strategy, risk-factor modification with cessation of smoking and lipid-lowering therapy should be implemented in patients with prior CABG surgery. An aggressive lipid-lowering strategy that targets a low-density lipoprotein level of less than 90 mg/L can be effective in reducing recurrent ischemic events and the need for subsequent revascularization procedures.

Recommendations for PCI With Prior CABG

Class I

1. Patients with early ischemia (usually within 30 days) after CABG. (*Level of Evidence: B*)

Class IIa

1. Patients with ischemia occurring 1 to 3 years postoperatively and preserved LV function with discrete lesions in graft conduits. (*Level of Evidence: B*)
2. Disabling angina secondary to new disease in a native coronary circulation. (If angina is not typical, the objective evidence of ischemia should be obtained.) (*Level of Evidence: B*)
3. Patients with diseased vein grafts >3 years following CABG. (*Level of Evidence: B*)

Class III

1. PCI to chronic total vein graft occlusions. (*Level of Evidence: B*)
2. Patients with multivessel disease, failure or multiple SVGs, and impaired LV function. (*Level of Evidence: B*)

E. Use of Adjunctive Technology (Intracoronary Ultrasound Imaging, Flow Velocity, and Pressure)

The limitations of coronary angiography for diagnostic and interventional procedures can be reduced by employing adjunctive technology of intracoronary ultrasound imaging, flow velocity, and pressure. Information obtained from the adjunctive modalities of intravascular imaging and physiology can improve PCI methods and outcomes.

1. Intravascular Ultrasound Imaging (IVUS)

IVUS is not necessary for all stent procedures. The results of the French Stent Registry study of 2900 patients treated without coumadin and without IVUS reported a subacute closure rate of 1.8%. In the STARS trial, a subacute closure rate of 0.6% in patients having optimal stent implantation supports the approach that IVUS does not appear to be required routinely in all stent implantations. However, the use of IVUS for evaluating results in high-risk procedures (i.e., those patients with multiple stents, impaired TIMI grade flow or coronary flow reserve, and marginal angiographic appearance) appears warranted.

In the context of published data and growing clinical experience, the Writing Committee has modified prior recommendations for the use of IVUS as follows.

Recommendations for Coronary Intravascular Ultrasound

Class IIa

1. Assessment of the adequacy of deployment of coronary stents, including the extent of stent apposition and determination of the minimum luminal diameter within the stent. (*Level of Evidence: B*)
2. Determination of the mechanism of stent restenosis (inadequate expansion vs. neointimal proliferation) and to enable selection of appropriate therapy (plaque ablation vs. repeat balloon expansion). (*Level of Evidence: B*)
3. Evaluation of coronary obstruction at a location difficult to image by angiography in a patient with a suspected flow-limiting stenosis. (*Level of Evidence: C*)
4. Assessment of a suboptimal angiographic result following PCI. (*Level of Evidence: C*)
5. Diagnosis and management of coronary disease following cardiac transplantation. (*Level of Evidence: C*)
6. Establish presence and distribution of coronary calcium in patients for whom adjunctive rotational atherectomy is contemplated. (*Level of Evidence: C*)
7. Determination of plaque location and circumferential distribution for guidance of directional coronary atherectomy. (*Level of Evidence: B*)

Class IIb

1. Determine extent of atherosclerosis in patients with characteristic anginal symptoms and a positive functional study with no focal stenoses or mild CAD on angiography. (*Level of Evidence: C*)
2. Preinterventional assessment of lesional characteristics and vessel dimensions as a means to select an optimal revascularization device. (*Level of Evidence: C*)

Class III

1. When angiographic diagnosis is clear and no interventional treatment is planned. (*Level of Evidence: C*)

2. Coronary Flow Velocity and Coronary Vasodilatory Reserve

Coronary flow velocity reserve (CVR), the ratio of hyperemic to basal flow, reflects flow resistance through the epicardial

artery and the corresponding myocardial bed. For lesion assessment, a normal CVR indicates a nonphysiologically significant stenosis. An abnormal CVR indicates that the stenosis in the epicardial artery is significant when the microcirculation is normal, confirmed by measuring rCVR. Several studies report that deferring PCI of non-flow-limiting lesions is safe, with <10% rate of lesion progression.

3. Coronary Artery Pressure and Fractional Flow Reserve

Fractional flow reserve (FFR) of the myocardium is the ratio of distal coronary pressure to aortic pressure measured during maximal hyperemia, which represents the fraction of normal blood flow through the stenotic artery. The normal FFR value for all vessels under all hemodynamic conditions, regardless of the status of microcirculation is 1.0. FFR values <0.75 are associated with abnormal stress tests. FFR does not use measurements in a reference vessel and is thought to be epicardial lesion-specific.

Reports indicate that a physiologic assessment can determine whether PTCA alone has achieved a satisfactory result with 6-month outcome equivalent to that reported with elective stenting. The DEBATE trial in 224 patients found that when a final diameter stenosis <35% and an excellent physiologic result (CVR >2.5) were obtained after PTCA (44/224 patients), the intermediate-term (6 months) target lesion revascularization and angiographic restenosis rates were 16%. Similar data have been reported for FFR. The application of coronary physiologic adjunctive modalities can facilitate decision making for moderate lesions, the appropriateness of PTCA, and the use of provisional stenting.

Recommendations for Intracoronary Physiologic Measurements (Doppler Ultrasound, FFR)

Class IIa

1. Assessment of the physiological effects of intermediate coronary stenoses (30 to 70% luminal narrowing) in patients with anginal symptoms. Coronary pressure or Doppler velocimetry may also be useful as an alternative to performing noninvasive functional testing (e.g., when the functional study is absent or ambiguous) to determine whether an intervention is warranted. (*Level of Evidence: B*)

Class IIb

1. Evaluation of the success of percutaneous coronary revascularization in restoring flow reserve and to predict the risk of restenosis. (*Level of Evidence: C*)
2. Evaluation of patients with anginal symptoms without an apparent angiographic culprit lesion. (*Level of Evidence: C*)

Class III

1. Routine assessment of the severity of angiographic disease in patients with a positive, unequivocal non-invasive functional study. (*Level of Evidence: C*)

VI. Management of Patients Undergoing PCI

A. Experience With New Technologies

The introduction of coronary stents and atherectomy devices has broadened the scope of patients that can be approached by PCI beyond those that could be safely treated by PTCA alone.

1. Acute Results

Significant reduction in the acute complication rate for PTCA has resulted from the adjunctive use of GP receptor IIb/IIIa blockers, which have been shown to reduce abrupt closure and periprocedural MI rates compared to placebo. Improved acute outcomes (in terms of abrupt closure rates and reduced target lesion residual diameter stenosis) have also been seen with the use of coronary stents, DCA, and adjunctive rotational atherectomy.

2. Late-Term Results

PCI devices offer the possibility of lower restenosis compared to PTCA in the native coronary circulation. Lower restenosis rates have been demonstrated for balloon-expandable slotted tubular stents in large (≥ 3 mm) native coronary arteries but are variable depending on lesion length for SVG lesions. Initial trials of DCA showed no benefit compared to PTCA for elective single-lesion treatment. Despite the improvement in acute results seen for rotational atherectomy and excimer laser, there is no evidence that these devices improve the late outcomes in lesions than can be feasibly treated by PTCA or stenting alone.

B. Antiplatelet and Antithrombotic Therapies and Coronary Angioplasty

1. Aspirin, Ticlopidine, Clopidogrel

Aspirin reduces the frequency of ischemic complications after coronary angioplasty. Although the minimum effective aspirin dosage in the setting of coronary angioplasty has not been established, an empiric dose of aspirin, 80 to 325 mg, given at least 2 h before PCI is generally recommended. While other antiplatelet agents have similar antiplatelet effects to aspirin, only the thienopyridine derivatives, ticlopidine and clopidogrel, have been routinely used as alternative antiplatelet agents in aspirin-sensitive patients during coronary angioplasty.

Ticlopidine has a number of important side effects. The most severe side effect is severe neutropenia, occurring in approximately 1% of patients. Clopidogrel, 300 mg loading dose followed by 75 mg daily, may be used as an alternative to ticlopidine in patients undergoing stent placement. A number of nonrandomized trials and a randomized trial have failed to show a difference in the clinical outcomes among patients treated with ticlopidine and clopidogrel after stent placement. A small number of cases of thrombocytopenia purpura have been reported in patients treated with clopidogrel; therefore, patients should be monitored during treatment for occurrence of this untoward effect.

2. GP IIb/IIIa Inhibitors

The binding of fibrinogen and other adhesive proteins to adjacent platelets by means of the GP IIb/IIIa receptor serves as the "final common pathway" of platelet-thrombus formation and can be effectively attenuated by GP IIb/IIIa antagonists. These agents have reduced the frequency of ischemic complications after coronary angioplasty.

Based on the numerous trials to date (Fig. 1), intravenous GP IIb/IIIa receptor inhibitors should be considered in patients undergoing coronary angioplasty, particularly those with unstable angina or with other clinical characteristics of

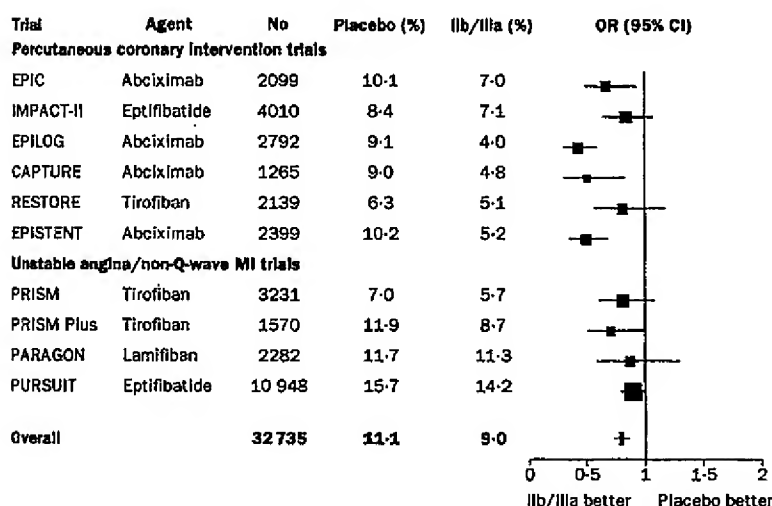


Figure 1. Death or nonfatal MI outcomes at 30 days in 10 randomized, placebo-controlled trials of GP IIb/IIIa blockers. Data and acronyms from references 30–39 (these numbers coincide with references in the original article). Risk ratio with 95% CI, size of RR box being proportional to total sample size. Frequency of death or nonfatal MI in columns 4 and 5. Overall (all 10 trials) benefit of GP IIb/IIIa blockade highly significant (RR = 0.79 [95% CI 0.73–0.85; $p < 10^{-9}$]). GP = glycoprotein; OR = odds ratio; CI = confidence interval; MI = myocardial infarction. Reproduced with permission from Topol EJ, et al. *Lancet* 1999;353:227–31.

high-risk. There is no consistent evidence that the GP IIb/IIIa inhibitors reduce the frequency of late restenosis in the nondiabetic patient. In EPISTENT, diabetic patients who received abciximab therapy in conjunction with stent deployment had a 51% reduction in target-vessel revascularization at 6 months. This trial is the only one that has shown a reduction in target-vessel revascularization in the diabetic group. It will be important to determine if supporting evidence is found from other trials using this agent and other GP IIb/IIIa antagonists.

3. Heparin

Heparin is an important component for PCI, despite dosing uncertainties and an unpredictable therapeutic response with the unfractionated preparation. Higher levels of anticoagulation with heparin are roughly correlated with therapeutic efficacy in the reduction of complications during coronary angioplasty, albeit at the expense of bleeding complications at very high levels of heparin dosing. It appears that weight-adjusted heparin dosing may provide a clinically superior anticoagulation method over fixed heparin dosing, although definitive studies are lacking.

Some patients with unstable angina are treated with low-molecular-weight heparin (LMWH) prior to coronary angioplasty. Anticoagulation monitoring is not routinely possible with LMWH, and conventional dosages of unfractionated heparin are currently recommended. Conventional ACT monitoring methods may underestimate the true degree of periprocedural anticoagulation with LMWH. Use of LMWH as the sole anticoagulant during PCI is not supported at this time in the absence of absolute or relative contraindications to unfractionated heparin, although data from clinical trials of these agents administered alone or in conjunction with GP IIb/IIIa blockade are forthcoming.

In those patients who do not receive GP IIb/IIIa inhibitors, sufficient unfractionated heparin should be given during

coronary angioplasty to achieve an ACT of 250 to 300 s with the HemoTec device and 300 to 350 s with the Hemochron device.

The unfractionated heparin bolus should be reduced to 50 to 70 IU/kg when GP IIb/IIIa inhibitors are given in order to achieve a target ACT of 200 s using either the HemoTec or Hemochron device.

C. Post-PCI Management

Following PCI, in-hospital care should focus on monitoring the patient for recurrent myocardial ischemia, achieving hemostasis at the catheter insertion site, and detecting and preventing contrast-induced renal failure. Attention should also be directed toward implementing appropriate secondary atherosclerosis prevention programs. The patient should understand and adhere to recommended medical therapies and behavior modifications known to reduce subsequent morbidity and mortality from coronary heart disease.

Most patients can be safely discharged from the hospital within 24 h after an uncomplicated elective PCI. Special skilled nursing units have been developed by many institutions to facilitate post-PCI management. Specific protocols for sheath removal, continuation of anticoagulation or antiplatelet therapies, and observation for recurrent myocardial ischemia/infarction and contrast-induced renal failure are of particular assistance in ensuring appropriate outcomes during this period. Pilot studies suggest that selected patients may be discharged on the same day after PCI especially when the procedure is performed by the percutaneous radial or brachial approach. However, confirmation by larger studies is necessary prior to widespread endorsement of this strategy.

1. Post-Procedure Evaluation of Ischemia

After PCI, chest pain may occur in as many as 50% of patients. ECG evidence of ischemia identifies those with significant risk for acute vessel closure. When angina pectoris

or ischemic ECG changes occur after PCI, the decision to proceed with further interventional procedures, CABG surgery, or medical therapy should be individualized based on factors such as hemodynamic stability, amount of myocardium at risk, and the likelihood that the treatment will be successful.

Patients with renal dysfunction and diabetes should be monitored for contrast-induced nephropathy. In addition, those patients receiving higher contrast loads or a second contrast load within 72 h should have renal function assessed. Whenever possible, nephrotoxic drugs (certain antibiotics, nonsteroidal anti-inflammatory agents, and cyclosporine) and metformin (especially in those with pre-existing renal dysfunction) should be withheld for 24 to 48 h prior to performing PCI and for 48 h afterwards.

2. Risk-Factor Modifications

All patients should be instructed about necessary behavior and risk-factor modification and the appropriate medical therapies for the secondary prevention of atherosclerosis prior to leaving the hospital. The interventional cardiologist should emphasize the importance of these measures directly to the patient as failure to do so may suggest that secondary prevention therapies are not necessary. The interventional cardiologist should interact with the primary care physician to assure that necessary secondary prevention therapies are initiated and maintained. Secondary prevention measures are an essential part of long-term therapy because they can reduce future morbidity and mortality associated with the atherosclerotic process.

Depending on the risk factors and contraindications present, advice should include aspirin therapy, hypertensive control, diabetic management, aggressive control of serum lipids to a target LDL goal <100 mgm/dl following AHA guidelines, abstinence from tobacco use, weight control, regular exercise, and ACE inhibitor therapy as recommended in the AHA/ACC consensus statement on secondary prevention.

3. Exercise Testing After PCI

Although restenosis remains the major limitation of PCI, symptom status is an unreliable index to development of restenosis with 25% of asymptomatic patients documented as having ischemia on exercise testing.

Because myocardial ischemia, whether painful or silent, worsens prognosis, some authorities have advocated routine testing. However, the ACC/AHA practice guidelines for exercise testing favor selective evaluation in patients considered to be at particularly high risk (e.g., patients with decreased LV function, multivessel CAD, proximal left anterior descending disease, previous sudden death, diabetes mellitus, hazardous occupations, and suboptimal PCI results). The exercise ECG is an insensitive predictor of restenosis, with sensitivities ranging from 40 to 55%, significantly less than those obtainable with SPECT or exercise echocardiography. This lower sensitivity of the exercise ECG and its inability to localize disease limits its usefulness in patient management both before and after PCI. For those reasons, stress imaging is preferred to evaluate symptomatic patients after PCI. If the patient's exertional capacity is significantly limited, coronary angiography may be more expeditious to

evaluate symptoms of typical angina. Exercise testing after discharge is helpful for activity counseling and/or exercise training as part of cardiac rehabilitation. Neither exercise testing nor radionuclide imaging is indicated for the routine, periodic monitoring of asymptomatic patients after PCI without specific indications.

VII. Special Considerations

A. Ad-Hoc Angioplasty—PCI at the Time of Initial Cardiac Catheterization

Ad-hoc coronary intervention is PCI performed at the same time as diagnostic cardiac catheterization. Since the last revision of these Guidelines, there has been an increase in ad-hoc interventions with reported incidence ranging from 52 to 83%.

Ad-hoc coronary intervention is particularly suitable for patients with clinical evidence of restenosis 6 to 12 months following the initial procedure, patients undergoing primary angioplasty for MI, and patients with refractory unstable angina in need of urgent revascularization. Ad-hoc PCI should be performed only in a well-informed patient, particularly in the setting of single-vessel disease without morphologic features predictive of an adverse outcome, when it is clear that this treatment strategy is the best alternative. This committee endorses the recommendations from the Society for Cardiac Angiography and Interventions that ad-hoc PCI be individualized and not be a standard or required strategy for all patients.

B. PCI in Cardiac Transplant Patients

Although high procedural success can be achieved and PCI may be applied in a selected cardiac transplant population with comparable success and complication rates to the routine patient population, it remains unknown whether PCI prolongs allograft survival. Coronary stenting in cardiac allograft vascular disease has been performed in small numbers of patients with favorable results. Long-term survival effects remain under examination.

C. Management of Clinical Restenosis

Although atheroablation devices have been developed in an attempt to lower the second restenosis risk in patients, none has shown an incremental benefit over PTCA. It is recommended that patients who develop restenosis following an initially successful PTCA be considered for repeat PCI with stent placement. Factors that may influence this decision include the technical difficulty of the initial procedure, the potential for the lesion to be treated successfully with a stent, and the severity and extent of the restenotic process. Each time restenosis recurs, consideration should be given to alternate methods of revascularization, particularly CABG surgery, as well as continued medical therapy.

D. Restenosis After Stent Implantation (In-stent Restenosis)

Intracoronary vascular radiation for in-stent restenosis with either gamma or beta radiation is the most promising therapy for in-stent restenosis at this time, reducing the chance for repeat restenosis by other methods from 50 to 60% to 25 to

35%. In the absence of vascular radiation for in-stent restenosis, there appears to be little difference in outcome between angioplasty alone as compared to combination with ablative techniques.

E. Cost-Effectiveness Analysis for PCI

While there is no established cost-effectiveness ratio threshold, cost-effectiveness ratios of <\$20,000 per QALY (such as seen in the treatment of severe diastolic hypertension or cholesterol lowering in patients with ischemic heart disease) are considered highly favorable and consistent with well accepted therapies.

In patients with severe angina, normal LV function, and single-vessel disease of the left anterior descending artery, the cost-effectiveness ratio for PTCA, directional coronary atherectomy, or coronary stenting that can be expected to provide >90% success rate with <3% major acute complication rate is very favorable (<\$20,000 per QALY) compared to medical therapy. In patients with 3-vessel coronary disease who have comorbidities that increase operative risk for CABG surgery, PCI that is felt to be safe and feasible is reasonably acceptable (\$20,000 to \$60,000 per QALY). In patients in the post-MI setting, a strategy of routine, nonsymptom-driven coronary, angiography and PCI performed for critical (>70% diameter stenosis) culprit coronary lesions amenable to PTCA or stenting has been proposed to be reasonably cost-effective in many subgroups.

In patients with symptomatic angina or documented ischemia and 3-vessel coronary disease, for which bypass surgery can be expected to provide full revascularization and an acute complication rate of less than 5%, the cost-effectiveness of PCI is not well established. Although PTCA for 2- and 3-vessel coronary disease appears to be as safe, but initially less expensive, than CABG surgery, the costs of PTCA converge towards the higher costs of bypass surgery after 3 to 5 years. Thus, while PTCA or CABG surgery has been shown to be cost-effective when compared to medical therapy, there

is no evidence for incremental cost-effectiveness of PTCA over bypass surgery for 2- or 3-vessel coronary disease in patients who are considered good candidates for both procedures. For patients with 1- or 2-vessel coronary disease who are asymptomatic or have only mild angina, without documented left main disease, the estimated cost-effectiveness ratios for PCI are greater than \$80,000 per QALY compared with medical therapy, and are thus considered less favorable.

Because CEA research is new in the field of percutaneous coronary intervention, CEA results are limited. The Committee underscores the need for cost containment and careful decision making regarding the use of PCI strategies.

VIII. Future Directions

An exciting arena of active investigation relates to methods of distal protection of the coronary vascular bed during PCI. It is now recognized that distal embolization is an important contributor to complications in patients undergoing SVG intervention. Distal embolization is often due to dislodgement of large, macroparticles from the friable graft, rather than release of platelet-mediated aggregates. This complication can be prevented by the use of distal occlusion balloons, such as the PercuSurge Guardwire, or with the use of distal filters that trap the debris and remove it from the distal circulation. A number of filter devices are currently undergoing clinical evaluation, particularly in saphenous vein graft disease and during carotid intervention.

Restenosis has also remained a vexing problem, despite the benefits achieved with stent implantation. Novel therapies have been developed, such as the application of therapeutic ultrasound, photodynamic therapy, and systemic administration of the anti-inflammatory agent tranilast. An area of active investigation involves the use of balloon-expandable stents coated with rapamycin, paclitaxol, or its derivative. The local delivery of these agents has shown promise in early clinical trials, and longer-term studies are currently underway.

Exhibit 10

AHA/ACC guidelines for preventing heart attack and death in patients with atherosclerotic cardiovascular disease: 2001 update: A statement for healthcare professionals from the American Heart Association and the American College of Cardiology

Sidney C. Smith, Jr, Steven N. Blair, Robert O. Bonow, Lawrence M. Brass, Manuel D. Cerqueira, Kathleen Dracup, Valentin Fuster, Antonio Gotto, Scott M. Grundy, Nancy Houston Miller, Alice Jacobs, Daniel Jones, Ronald M. Krauss, Lori Mosca, Ira Ockene, Richard C. Pasternak, Thomas Pearson, Marc A. Pfeffer, Rodman D. Starke, and Kathryn A. Taubert
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AHA/ACC Scientific Statement

AHA/ACC Guidelines for Preventing Heart Attack and Death in Patients With Atherosclerotic Cardiovascular Disease: 2001 Update

A Statement for Healthcare Professionals From the American Heart Association and the American College of Cardiology

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Since the original publication (in 1995) of the American Heart Association (AHA) consensus statement on secondary prevention, which was endorsed by the American College of Cardiology (ACC), important evidence from clinical trials has emerged that further supports the merits of aggressive risk reduction therapies for patients with atherosclerotic cardiovascular disease. As noted in that statement, aggressive risk factor management clearly improves patient survival, reduces recurrent events and the need for interventional procedures, and improves the quality of life for these patients.

The compelling evidence from recent clinical trials was the impetus to revise the 1995 guidelines (Table). As examples, the many lipid reduction trials have generated significant changes in the National Heart, Lung, and Blood Institute's Adult Treatment Panel III report. This report further defined target cholesterol levels, expanded indications for drug treatment, and initiated therapy earlier. Accumulating β -blocker data have resulted in broader indications for a larger patient group. The Heart Outcomes Prevention Evaluation (HOPE) trial has demonstrated the benefit of ACE inhibitor therapy in high-risk patients with cardiovascular disease without a history of an acute event. Further data from ongoing trials should provide insight into the potential benefits of treating lower risk patients with combined therapies. The Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events (CAPRIE) trial has provided evidence for clopidogrel benefit in

certain patients. Diabetes management recommendations have been updated to include recent guidelines from the American Diabetes Association for risk factor management of diabetics and the growing body of evidence showing diabetics at high risk for cardiovascular events. The Heart and Estrogen/progestin Replacement Study (HERS) documented that hormone replacement therapy is ineffective for secondary prevention. The writing group revising this document also considered other important trials and reports, and they are included in the selected reading list.

In the 6 years since the guidelines were first published, 2 other developments have made them even more important in clinical care: the aging of the population continues to expand the number of patients living with a diagnosis of cardiovascular disease (now estimated at 12.4 million), and the multiple studies of the actual use of these recommended therapies in appropriate patients, while showing slow improvement, have continued to support the discouraging conclusion that a large proportion of patients in whom therapies are indicated are not receiving those therapies in actual clinical practice. The AHA and ACC continue to urge that all medical care settings in which these patients are managed organize a specific plan to identify appropriate patients, provide practitioners with useful reminder clues based on the guidelines, and continuously assess the success achieved in providing all appropriate therapies to all of the patients who can benefit from them.

The American Heart Association makes every effort to avoid any actual or potential conflicts of interest that may arise as a result of an outside relationship or a personal, professional, or business interest of a member of the writing panel. Specifically, all members of the writing group are required to complete and submit a Disclosure Questionnaire showing all such relationships that might be perceived as real or potential conflicts of interest.

This statement was approved by the American Heart Association Science Advisory and Coordinating Committee in June 2001 and by the American College of Cardiology Board of Trustees in July 2001. A single reprint is available by calling 800-253-4636 (US only) or writing the American College of Cardiology, Educational Services, 9111 Old Georgetown Road, Bethesda, MD 20814-1699. Ask for reprint No. 71-0214. To purchase additional reprints: up to 999 copies, call 800-611-6083 (US only) or fax 413-665-2671; 1000 or more copies, call 214-706-1466, fax 214-691-6342, or e-mail pubauth@heart.org. To make photocopies for personal or educational use, call the Copyright Clearance Center, 978-750-8400.

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AHA/ACC Secondary Prevention for Patients With Coronary and Other Vascular Disease: 2001 Update

Goals	Intervention Recommendations		
Smoking: <u>Goal</u> complete cessation	Assess tobacco use. Strongly encourage patient and family to stop smoking and to avoid secondhand smoke. Provide counseling, pharmacological therapy, including nicotine replacement and bupropion, and formal smoking cessation programs as appropriate.		
BP control: <u>Goal</u> <140/90 mm Hg or <130/85 mm Hg if heart failure or renal insufficiency <130/80 mm Hg if diabetes	Initiate lifestyle modification (weight control, physical activity, alcohol moderation, moderate sodium restriction, and emphasis on fruits, vegetables, and low-fat dairy products) in all patients with blood pressure ≥ 130 mm Hg systolic or 80 mm Hg diastolic. Add blood pressure medication, individualized to other patient requirements and characteristics (ie, age, race, need for drugs with specific benefits) if blood pressure is not <140 mm Hg systolic or 90 mm Hg diastolic or if blood pressure is not <130 mm Hg systolic or 85 mm Hg diastolic for individuals with heart failure or renal insufficiency (<80 mm Hg diastolic for individuals with diabetes).		
Lipid management: <u>Primary goal</u> LDL <100 mg/dL	Start dietary therapy in all patients (<7% saturated fat and <200 mg/d cholesterol) and promote physical activity and weight management. Encourage increased consumption of omega-3 fatty acids. Assess fasting lipid profile in all patients, and within 24 hr of hospitalization for those with an acute event. If patients are hospitalized, consider adding drug therapy on discharge. Add drug therapy according to the following guide:		
	LDL <100 mg/dL (baseline or on-treatment) Further LDL-lowering therapy not required Consider fibrate or niacin (if low HDL or high TG)	LDL 100–129 mg/dL (baseline or on-treatment) Therapeutic options: Intensify LDL-lowering therapy (statin or resin*) Fibrate or niacin (if low HDL or high TG) Consider combined drug therapy (statin+fibrate or niacin) (if low HDL or high TG)	LDL ≥ 130 mg/dL (baseline or on-treatment) Intensify LDL-lowering therapy (statin or resin*) Add or increase drug therapy with lifestyle therapies
Lipid management: <u>Secondary goal</u> If TG ≥ 200 mg/dL, then non-HDL† should be <130 mg/dL	If TG ≥ 150 mg/dL or HDL <40 mg/dL: Emphasize weight management and physical activity. Advise smoking cessation. If TG 200–499 mg/dL: Consider fibrate or niacin <i>after</i> LDL-lowering therapy* If TG ≥ 500 mg/dL: Consider fibrate or niacin <i>before</i> LDL-lowering therapy* Consider omega-3 fatty acids as adjunct for high TG		
Physical activity: <u>Minimum goal</u> 30 minutes 3 to 4 days per week <u>Optimal</u> daily	Assess risk, preferably with exercise test, to guide prescription. Encourage minimum of 30 to 60 minutes of activity, preferably daily, or at least 3 or 4 times weekly (walking, jogging, cycling, or other aerobic activity) supplemented by an increase in daily lifestyle activities (eg, walking breaks at work, gardening, household work). Advise medically supervised programs for moderate- to high-risk patients.		
Weight management: <u>Goal</u> BMI 18.5–24.9 kg/m ²	Calculate BMI and measure waist circumference as part of evaluation. Monitor response of BMI and waist circumference to therapy. Start weight management and physical activity as appropriate. Desirable BMI range is 18.5–24.9 kg/m ² . When BMI ≥ 25 kg/m ² , goal for waist circumference is ≤ 40 inches in men and ≤ 35 inches in women.		
Diabetes management: <u>Goal</u> HbA _{1c} <7%	Appropriate hypoglycemic therapy to achieve near-normal fasting plasma glucose, as indicated by HbA _{1c} . Treatment of other risks (eg, physical activity, weight management, blood pressure, and cholesterol management).		
Antiplatelet agents/ anticoagulants:	Start and continue indefinitely aspirin 75 to 325 mg/d if not contraindicated. Consider clopidogrel 75 mg/d or warfarin if aspirin contraindicated. Manage warfarin to international normalized ratio=2.0 to 3.0 in post-MI patients when clinically indicated or for those not able to take aspirin or clopidogrel.		
ACE inhibitors:	Treat all patients indefinitely post MI; start early in stable high-risk patients (anterior MI, previous MI, Killip class II [S ₃ gallop, rales, radiographic CHF]). Consider chronic therapy for all other patients with coronary or other vascular disease unless contraindicated.		
β-Blockers:	Start in all post-MI and acute ischemic syndrome patients. Continue indefinitely. Observe usual contraindications. Use as needed to manage angina, rhythm, or blood pressure in all other patients.		

BP indicates blood pressure; TG, triglycerides; BMI, body mass index; HbA_{1c}, major fraction of adult hemoglobin; MI, myocardial infarction; and CHF, congestive heart failure.

*The use of resin is relatively contraindicated when TG >200 mg/dL.

†Non-HDL cholesterol=total cholesterol minus HDL cholesterol.

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KEY WORDS: AHA Scientific Statements ■ prevention ■ risk factors
■ atherosclerosis

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Exhibit 11

CYCLOOXYGENASES 1 AND 2

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ABSTRACT

Cyclooxygenase (COX), first purified in 1976 and cloned in 1988, is the key enzyme in the synthesis of prostaglandins (PGs) from arachidonic acid. In 1991, several laboratories identified a product from a second gene with COX activity and called it COX-2. However, COX-2 was inducible, and the inducing stimuli included pro-inflammatory cytokines and growth factors, implying a role for COX-2 in both inflammation and control of cell growth. The two isoforms of COX are almost identical in structure but have important differences in substrate and inhibitor selectivity and in their intracellular locations. Protective PGs, which preserve the integrity of the stomach lining and maintain normal renal function in a compromised kidney, are synthesized by COX-1. In addition to the induction of COX-2 in inflammatory lesions, it is present constitutively in the brain and spinal cord, where it may be involved in nerve transmission, particularly that for pain and fever. PGs made by COX-2 are also important in ovulation and in the birth process. The discovery of COX-2 has made possible the design of drugs that reduce inflammation without removing the protective PGs in the stomach and kidney made by COX-1. These highly selective COX-2 inhibitors may not only be anti-inflammatory but may also be active in colon cancer and Alzheimer's disease.

INTRODUCTION

Cyclooxygenase (COX) or prostaglandin H₂ synthase (PGHS) is the enzyme that catalyzes the first two steps in the biosynthesis of the prostaglandins (PGs)

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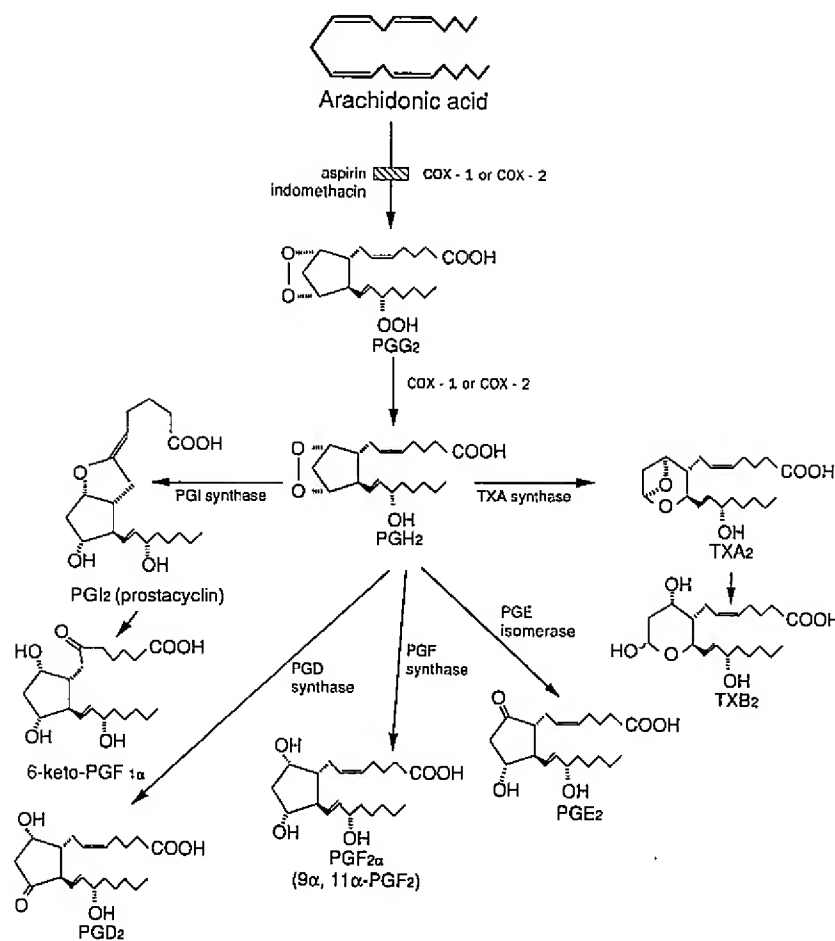


Figure 1 The arachidonic acid cascade.

from the substrate arachidonic acid (AA). These are the oxidation of AA to the hydroperoxy endoperoxide PGG_2 and its subsequent reduction to the hydroxy endoperoxide PGH_2 . The PGH_2 is transformed by a range of enzymes and nonenzymic mechanisms into the primary prostanoids, PGE_2 , $\text{PGF}_{2\alpha}$, PGD_2 , PGI_2 , and TXA_2 (Figure 1).

COX activity has long been studied in preparations from sheep seminal vesicles, and this enzyme was cloned by three separate groups in 1988 (1–3). The discovery of a second form of COX in the early 1990s was the most important

event in prostanoid biology in almost 20 years. Induction of this isoform, COX-2, by several stimuli associated with cell activation and inflammation assured the relevance of this finding to inflammatory disease in general. A clear sign of the therapeutic value of this discovery is that in the relatively short time of about five years, several highly effective anti-inflammatory agents and new therapeutic areas have become subjects for investigation. This review concentrates on those aspects in COX and PG research that have proved to be most relevant to the development of new anti-inflammatory drugs. The reader is referred for further reading to some recent reviews (4–7).

BIOCHEMISTRY AND CRYSTAL STRUCTURE OF COX-1 AND COX-2

Biochemical Comparisons

The inducible enzyme COX-2 is very similar in structure and catalytic activity to the constitutive COX-1. The biosynthetic activity of both isoforms can be inhibited by aspirin and other nonsteroid anti-inflammatory drugs (NSAIDs) (8). The inhibition by aspirin is due to the irreversible acetylation of the COX site of PGHS, leaving the peroxidase activity of the enzyme unaffected. In contrast to this irreversible action of aspirin, other NSAIDs such as ibuprofen or indomethacin produce reversible or irreversible inhibition by competing with the substrate AA for the active site of the enzyme. Both isoforms have a molecular weight of 71 K and are almost identical in length, with just over 600 amino acids, of which 63% are in an identical sequence. However, the human COX-2 gene at 8.3 kb is a small immediate early gene, whereas human COX-1 originates from a much larger 22-kb gene. The gene products also differ, with the mRNA for the inducible enzyme being approximately 4.5 kb and that of the constitutive enzyme being 2.8 kb (4, 5). The three-dimensional X-ray crystal structure of human or murine COX-2 (9, 10) can be superimposed on that of COX-1 (11); the residues that form the substrate binding channel, the catalytic sites, and the residues immediately adjacent are all identical except for two small variations. In these two positions, the same substitutions occur: Ile in COX-1 is exchanged for Val in COX-2 at positions 434 and 523 (the residues in COX-2 are given the same number as their equivalent amino acids in COX-1).

In spite of this structural identity, there are clear biochemical differences between the isoforms in substrate and inhibitor selectivity. For example, COX-2 will accept a wider range of fatty acids as substrates than will COX-1 (4). Thus, although both enzymes can utilize AA and dihomo- γ -linolenate equally well, COX-2 oxygenates other fatty acid substrates, such as eicosapentaenoic acid, γ -linolenic acid, α -linolenic acid, and linoleic acid more efficiently than does

COX-1. Also, COX-2 acetylated by aspirin on Ser 530 will still oxidize AA but to 15-HETE, whereas similarly acetylated COX-1 will not oxidize AA at all (12–14). In addition (see below), inhibitors will differentiate between COX-2 and COX-1 with over 1000-fold selectivity (6, 15).

Structural Comparisons

The biochemical differences between the two isoforms of the enzyme have been attributed to the changes resulting from the Ile/Val substitutions mentioned above. Supporting evidence is strongest from the work on COX-2-selective inhibitors; mutation of Ile 523 to Val in the COX-1 protein allows COX-2-selective inhibitors to bind and inhibit PGH₂ formation without altering the K_m for AA (16), and the reverse mutant of COX-2 in which Val 523 is exchanged for Ile shows inhibitor binding and selectivity profiles comparable to those of wild-type COX-1 (17, 18). The structural basis for this has been shown clearly in the crystal analyses of COX-2, which have used either the human (9) or the murine protein (10), each bound to a nonselective COX-1 or a selective COX-2 inhibitor. The smaller size of Val 523 allows the inhibitor access to a side pocket off the main substrate channel in COX-2—access that is denied sterically by the longer side chain of Ile in COX-1. Selective inhibitors of COX-2 do not bind to Arg 120, which is used by the carboxylic acid of the substrate AA and by the COX-1-selective or -nonselective NSAIDs, all of which are carboxylic acids (19, 20).

Kurumbail et al (10) have suggested that the other Val substitution in COX-2, at residue 434, also contributes to the opening of the side pocket. However, it is more likely that this variation controls the substrate selectivity of COX-2. This residue is closer to the acetyltable Ser 530, and the smaller bulk of Val could provide additional space in this region of the substrate channel to allow a greater range of substrates in native COX-2 and, in acetylated COX-2, to allow AA to “squeeze” past the acetylated Ser 530 into the catalytic site. Support for this suggestion can be deduced from the results obtained with the recently described double mutant of COX-1 with Ile523Val and His513Arg, which exhibited increased binding of selective COX-2 inhibitors and decreased binding of COX-1 inhibitors, as predicted, but did not show increased 15-HETE production after acetylation with aspirin (16).

Another striking structural difference between the isoforms, but of unknown significance, is the absence of a sequence of 17 amino acids from the N terminus and the insertion of a sequence of 18 amino acids at the C terminus of COX-2 in comparison to COX-1 (4, 5). This accounts for the different numbering for the analogous residues in the two isoforms (e.g. the acetyltable serine is Ser 530 in COX-1 but Ser 516 in COX-2). The C-terminal insert in COX-2 does not alter the last four amino acid residues, which in both proteins form the signal

for attachment to the membrane of the endoplasmic reticulum (ER) (21, 22). However, COX-2 is located on the nuclear membrane as well as on the ER (23–25), while COX-1 is found attached only to the membranes of the ER. The reason for this selective localization may lie in the different sequence of the C terminus. It is relevant that in the X-ray structural analysis of either isoform, the three-dimensional structures of the last 18 C-terminal residues in COX-1 and the last 30 residues in COX-2 were not resolved, implying a marked flexibility in this region of the proteins even in the crystalline form (10, 11).

Although emphasis has been placed here on the differences between isoforms, the extensive overall structural and biochemical similarity between COX-1 and COX-2 must be reiterated. Both use the same endogenous substrate, AA, and form the same product by the same catalytic mechanism. Their major difference lies in their pathophysiological functions.

PHYSIOLOGICAL AND PATHOLOGICAL FUNCTIONS OF COX-1 AND COX-2

Chronic inflammation is an excellent example of a disease that represents a malfunction of normal host defense systems. Thus, rather than classifying PG biosynthesis into physiological and pathological, it may be better to use the classification applied to the COX isoforms: either constitutive or induced. COX-1 activity is constitutive, present in nearly all cell types at a constant level; COX-2 activity is normally absent from cells, and when induced, the protein levels increase and decrease in a matter of hours after a single stimulus (4, 5).

The main reason for labeling COX-1 and COX-2 as physiological and pathological, respectively, is that most of the stimuli known to induce COX-2 are those associated with inflammation, for example, bacterial lipopolysaccharide (LPS) and cytokines such as interleukin (IL)-1, IL-2, and tumor necrosis factor (TNF)- α . The anti-inflammatory cytokines, IL-4, IL-10, and IL-13, will decrease induction of COX-2, as will the corticosteroids (4, 6, 26). The physiological roles of COX-1 have been deduced from the deleterious side effects of NSAIDs, which while inhibiting PG biosynthesis at inflammatory sites, also inhibit constitutive biosynthesis. Thus, COX-1 provides PGs in the stomach and intestine to maintain the integrity of the mucosal epithelium and its inhibition leads to gastric damage, hemorrhage, and ulceration.

The Stomach

In most species, including humans, cytoprotective PGs in the stomach are synthesized by COX-1, although small quantities of COX-2 are also expressed constitutively (27). It has always been assumed that the cytoprotective role of PGs (e.g. prostacyclin; PGI₂) in the stomach is largely due to their vasodilating

properties, enhancing mucosal blood flow. Thus, PGs produced by COX-1 confer protection on the epithelial cells of the crypts of Lieberkühn in the ileum of irradiated mice. Radiation injury results in a decrease in the number of surviving crypt stem cells. These numbers were further reduced by the administration of indomethacin to the irradiated mice but not of a selective COX-2 inhibitor. Since the presence of COX-1 was demonstrated in the epithelial cells of the crypts of nonirradiated mice and in the regenerating crypt epithelium of irradiated animals, PGs produced by COX-1 are the most likely to promote crypt stem cell survival and proliferation (28). The increased mucosal damage caused by indomethacin is also likely to be due to inhibition of COX-1. Interestingly, COX-2 mRNA levels were raised in human gastric adenocarcinoma tissues compared with those in normal specimens of gastric mucosal tissue. COX-1 mRNA levels were not elevated in the carcinoma (29).

The Kidney

PGs do not maintain normal renal blood flow, but PG production becomes important in maintaining blood flow of the compromised kidney (30). Maintenance of normal kidney function is dependent on PGs both in animal models of disease states and in patients with congestive heart failure, liver cirrhosis, or renal insufficiency. Patients are therefore at risk of renal ischemia when PG synthesis is reduced by chronically administered NSAIDs. Those kidney cells that synthesize PGs contain mostly COX-1, but low levels of COX-2 mRNA have also been detected (31). Cultured rat mesangial cells increase their production of PGI₂ and PGE₂ after induction of COX-2 with cytokines or growth factors (32). The PGI₂ formed by mesangial cells may directly stimulate renin secretion as a feedback control for inhibition of salt reabsorption. Up-regulation of COX-2 expression has been observed in the macula densa, following salt deprivation (31).

The Platelet

In the platelet, the only isoform detectable is COX-1, and loss of AA-induced platelet aggregation is not only a well-established side effect of NSAID treatment, but also the therapeutic aim of the "half an aspirin a day" prophylaxis against thromboembolic disease (33). This prophylaxis is achieved through inhibition of COX-1, which leads to decreased production of thromboxane A₂ (TXA₂). Prostacyclin production in endothelial cells is also decreased, but the COX-1 there regenerates so that PGI₂ synthesis is reestablished. However, platelets do not form new enzyme, and TXA₂ synthesis is irreversibly inhibited for their lifetime of 8–10 days in the circulation. In addition, aspirin acetylates COX-1 of the platelets in the presystemic circulation before it reaches the general circulation. As it passes through the liver, up to 50% of the aspirin is

deacetylated and it becomes diluted further when joining the rest of the venous blood. In humans, aspirin blocks COX activity in platelets within an hour of oral administration (8). This results in inhibition of platelet function for several days after a single dose of aspirin. Dose regimens from 25 to 325 mg a day have been suggested, but a consensus of opinion now recommends 75 mg a day (34).

Gestation and Parturition

PGs are important for inducing uterine contractions during labor. NSAIDs such as indomethacin will thus delay premature labor by inhibiting this production of PGs (35). Expression of COX-1 is much greater than that of COX-2 in fetal hearts, kidneys, lungs, and brains, as well as in the decidual lining of the uterus (35,36). Constitutive COX-1 in the amnion could also contribute PGs for the maintenance of a healthy pregnancy (37). In human amnion cells, human chorionic gonadotrophin stimulates the expression of the COX-1 gene and increases mRNA and COX-1 protein levels (38).

Both COX-1 and COX-2 are expressed in the uterine epithelium at different times in early pregnancy and may be important for implantation of the ovum and in the angiogenesis needed for establishment of the placenta (39). PGs originating from COX-2 may play a role in the birth process, since COX-2 mRNA in the amnion and placenta increases substantially immediately before and after the start of labor (36). Glucocorticoids, EGF, IL-1 β , and IL-4 all stimulate COX-2 production in human amnion cells (40,41), and glucocorticoids can cause premature labor in pregnant sheep, possibly by inducing progesterone-metabolizing enzymes that reduce progesterone levels below those needed to maintain pregnancy (42). Preterm labor could be caused by an intrauterine infection resulting in release of endogenous factors that increase PG production by up-regulating COX-2 (41). Selective inhibitors of COX-2 reduce PG synthesis in isolated fetal membranes and should be useful in delaying premature labor without the side effects of indomethacin (35).

Gene Deletion Studies

In contrast to the analysis based essentially on the effects of inhibition of COX-1 with NSAIDs in adults are the results from molecular biology's equivalent of ablation, the gene knockout. Mice with the COX-1 gene disrupted lack mRNA, protein, and enzyme activity of COX-1 (43). Their platelets are unresponsive to AA, but they exhibit no gastric or intestinal ulcers nor any renal dysfunction. The absence of spontaneous gastric bleeding or erosions in these knockout mice leads to the conclusion that other cytoprotective mechanisms, such as the synthesis of nitric oxide or calcitonin gene-related peptide (CGRP), take over in the absence of the PGs. The lack of renal pathology confirms previous findings that NSAIDs only cause dysfunction in already compromised kidneys.

However, PGs synthesized by COX-1 are apparently essential for the survival of fetuses, since the majority of offspring born to homozygous COX-1 knockout mice did not survive (43).

The COX-2 knockout strain of mouse yielded similarly unexpected results (44, 45). These knockout mice showed unchanged responses to acute experimental inflammation induced by AA or phorbol ester. The female mice were infertile, for they did not ovulate. Furthermore the COX-2 knockout mice had serious renal developmental deficiencies postpartum and a consequently short life span. These results would imply a constitutive role for COX-2 in ovulation, of which there is already some indication (46), but would appear to deny the relevance of COX-2 to inflammation, in contrast to the ample evidence of the presence of COX-2 protein and the effects of COX-2 inhibition on inflammatory events. However, in the tests reported, responses of the ears of the mice to AA or phorbol ester were measured between one and four hours later and could well have been due to PGs formed by COX-1. These studies also raise the possibility of the teratological potential of COX-2 inhibition. The questions raised by the discrepancies between the results from knockout mice and the predictions from the behavior of normal mice need to be resolved (6, 47, 48), but they do suggest a general caution in the interpretation of results from knockout models as indications of the physiological roles of mediators in adult animals.

COX-1 AND COX-2 IN THE CNS

COX-1 is distributed in neurones throughout the brain, but it is most prevalent in forebrain, where PGs may be involved in complex integrative functions, such as modulation of the autonomic nervous system and sensory processing (49, 50). COX-2 is expressed constitutively in only a few organs and one of those is the brain. This expression is restricted to certain parts of the CNS, notably the cortex, hippocampus, hypothalamus, and spinal cord (50, 51). It is the predominant isoform in the brains of neonate pigs (52) and in the spinal cord of the rat (53), while human brain tissues contain equal amounts of mRNA for COX-1 and COX-2 (54).

Nerve Transmission

The most interesting feature of COX-2 in the CNS is that the enzyme is up-regulated by normal or by abnormal (convulsive) nerve activity (49). Furthermore, COX-2 protein or mRNA was detected in neurones as well as in the nonneuronal cells of the CNS (49–51). These findings suggest a role for PGs in CNS transmission and raise the possibility that selective COX-2 inhibitors may modulate CNS function. This would be especially relevant for those COX-2 inhibitors that lack an acidic group and may, therefore, pass the blood brain

barrier. The major PGs in the CNS of most mammalian species—including humans, monkeys, and rats—are PGE₂ and PGD₂. In neonatal rats, PGD₂ synthase was found in the neurons of the brain, but in adult animals, the neuronal enzyme had disappeared, and PGD₂ synthase was now located in the nonneuronal cells lining the CNS (meninges and choroid plexus) and also within the cerebral spinal fluid (CSF), as postnatal development progresses (55). Thus in the adult rat, the neurones express COX-2 and synthesize PGH₂, but the subsequent metabolism to PGD₂ must take place in the nonneuronal cells or in the extracellular space. The unusual formation of this important sleep-inducing PG needs more evaluation before its functional importance can be assessed.

Fever

It has been postulated that PGE₂ produced in the organum vasculosum laminae terminalis (OVLT) generates neuronal signals that activate the thermoregulatory center in the preoptic area of the anterior hypothalamus, which is situated close to the OVLT (56). PGE₂ synthesis is stimulated by cytokines such as IL-1, which are released by the actions of pyrogens such as LPS. Although the expression of COX-2 in the CNS is increased after LPS, the induction is not in neurones but in the endothelium of cranial blood vessels and in the microglia (57, 58). Furthermore, COX-2 in rat telencephalic neurones is induced by LPS but inhibited by urethane anesthesia without modifying the febrile response to LPS (59). Thus, it is clear that PGE₂ involved in the febrile response derives from COX-2 induced in nonneuronal cells, probably endothelial cells of the blood vessels perfusing the hypothalamus.

Hyperalgesia

Another apparently central effect of PGs, considered to be mediated peripherally, is pain or, more accurately, hyperalgesia. Although the thalamus and other higher nuclei of the CNS associated with pain pathways are not rich in constitutive or induced COX-2, the spinal cord may be where the nociceptive process is most influenced by COX-2. For some time, it has been known that during inflammatory pain, PGs are generated at the peripheral terminals of sensory neurones and cause hyperalgesia (60, 61). This is accompanied by production of pro-inflammatory cytokines (IL-1, IL-8, and TNF- α) and most probably by induction of COX-2 in inflammatory cells, if not in the nerve terminals themselves (61–63). Intrathecal administration of PGE₂ into conscious rats or mice induced hyperalgesia (64, 65). Moreover, ibuprofen, aspirin, ketorolac, indomethacin, or NS398 were potent analgesics in the formalin test when given intrathecally to rats (66, 67), suggesting an additional role for PGs in nociceptive processing in the spinal cord in this model of analgesia. More directly, an increase in mRNA (68) or immunoreactive staining (53) for COX-2, but

not for COX-1, in rat spinal cord resulted from inflammatory injury to the paw. However the rise in COX-2 mRNA was transient, lasting about 6–12 h, whereas inflammation and hyperalgesia persisted for several days (68). The effect of a noninflammatory stimulus—repetitive, low-frequency electrical stimulation of the sural nerve—which normally leads to a potentiation of the withdrawal reflex, was also susceptible to inhibition by indomethacin and SC58125, a selective COX-2 inhibitor, implying a mediation of PGs generated by COX-2 in another spinal response to a nociceptive stimulus (69). In a preliminary report, COX-2 was found to be localized to discrete laminae of rat spinal cord, in neuronal and nonneuronal tissue (53). It will be important to establish whether COX-2 in the spinal cord is in neurones or in nonneuronal cells and also which compartment shows induction in hyperalgesic conditions.

COX-2 IN THE NUCLEUS

Another possible function of COX-2, but not of COX-1, stems from its perinuclear location (22–25). The traditional view of the PGs is as extracellular and intercellular messengers acting on (the G-protein-linked, seven transmembrane type) cell membrane receptors and implying an export of PGs from the cell for their function to be realized. Indications for an intracellular function of PGs, which for many years were considered indirect (70), have been strengthened by the demonstration that PGI_2 derivatives are potent ligands for the nuclear peroxisome proliferator-activated receptor- γ (PPAR- γ) (71, 72). This receptor is in the retinoid X receptor (RXR) heterodimer nuclear receptor family, a diverse group including the retinoid and thyroid nuclear receptors, all acting as transcription factors for genomic DNA (73). An action on a nuclear receptor might account for the cytostatic effects of PGI_2 in transformed cell lines (74) and, in another context, form the link between COX-2 activity and the progression of precancerous epithelial cells to fully malignant phenotypes (see below) (75).

Another intriguing finding was the increase in Bcl-2 protein following COX-2 transfection and action in epithelial cells (76). The amount of this protein was decreased by treatment with a NSAID (sulindac), which also increased the apoptotic rate in the cell cultures. Although the link between PG production and Bcl-2 synthesis has not been elucidated, it is clear that in these cells, PGs synthesized by COX-2 influence another nuclear event, apoptosis. The pleiotropic character of the PGs and thus of COX was illustrated by the contrasting results in chick embryo fibroblasts where NSAIDs, in admittedly high concentrations, induced COX-2 protein and apoptosis (77). Whatever the final resolution of these paradoxes, the effects of PGs on cell growth and death have a topological justification in the perinuclear locus of COX-2.

COX-2 AND INFLAMMATION

The discovery and characterization of COX-2 have answered some long-standing puzzles and created new and fascinating problems in biology. They have also solved one problem in therapeutics—how to suppress inflammation without the side effects of the present range of NSAIDs. These side effects—gastrointestinal ulceration and bleeding, renal damage, and platelet dysfunction—were accepted as inevitable consequences of the inhibition of COX activity required to prevent synthesis of PGs in inflammatory conditions such as rheumatoid or osteo-arthritis. Now with COX-2 clearly associated with inflammation but not with the physiological synthesis of PGs, selective inhibitors of COX-2 offered the possibility of inhibition of inflammatory PGs without affecting PGs generated by COX-1 in the stomach, kidney or platelet: “an aspirin without ulcers.” This possibility has generated a great deal of effort and a considerable degree of success in pharmaceutical research.

Selective Inhibition of COX-2

The design of selective inhibitors would logically follow from the extensive structural analysis of the two isoforms of COX. However, the first generation of selective COX-2 inhibitors came from animal models in which compounds were sought that were potent anti-inflammatory agents with minimal side effects on the stomach. Nimesulide, etodolac, and meloxicam were discovered in this way, and they have all reached the market. Now, we know that they are selective for COX-2 rather than COX-1 (Table 1). Interestingly, the highly selective COX-2 inhibitors now in Phase III clinical trials were developed from the molecular variations of nimesulide, which has been marketed for 20 years (78). Nimesulide had been regarded as an aberrant example of an NSAID with good in vivo potency in inflammatory models but weak inhibition in vitro of the COX preparation available at the time. This preparation was derived from seminal vesicles and was almost certainly pure COX-1. Later evaluation of nimesulide, etodolac, and meloxicam (Figure 2) against COX-1 and COX-2 preparations confirmed their selectivity for COX-2 in contrast to the nonselective or COX-1-selective NSAIDs such as diclofenac, indomethacin, piroxicam, or naproxen. The selectivity ratios of inhibition for these COX-2 inhibitors were highly variable (Table 2), largely as a result of the variety of experimental conditions used in the assays (6), but always ranged from 10- to 100-fold selectivity for COX-2. Large-scale clinical trial results of one of this group (meloxicam) clearly show that severe gastric damage is significantly less than that caused by diclofenac or piroxicam, reinforcing the whole concept (79).

The newer compounds specifically designed by medicinal chemists as COX-2 inhibitors, such as SC58125 and L-745,337 (Figure 2), are more selective,

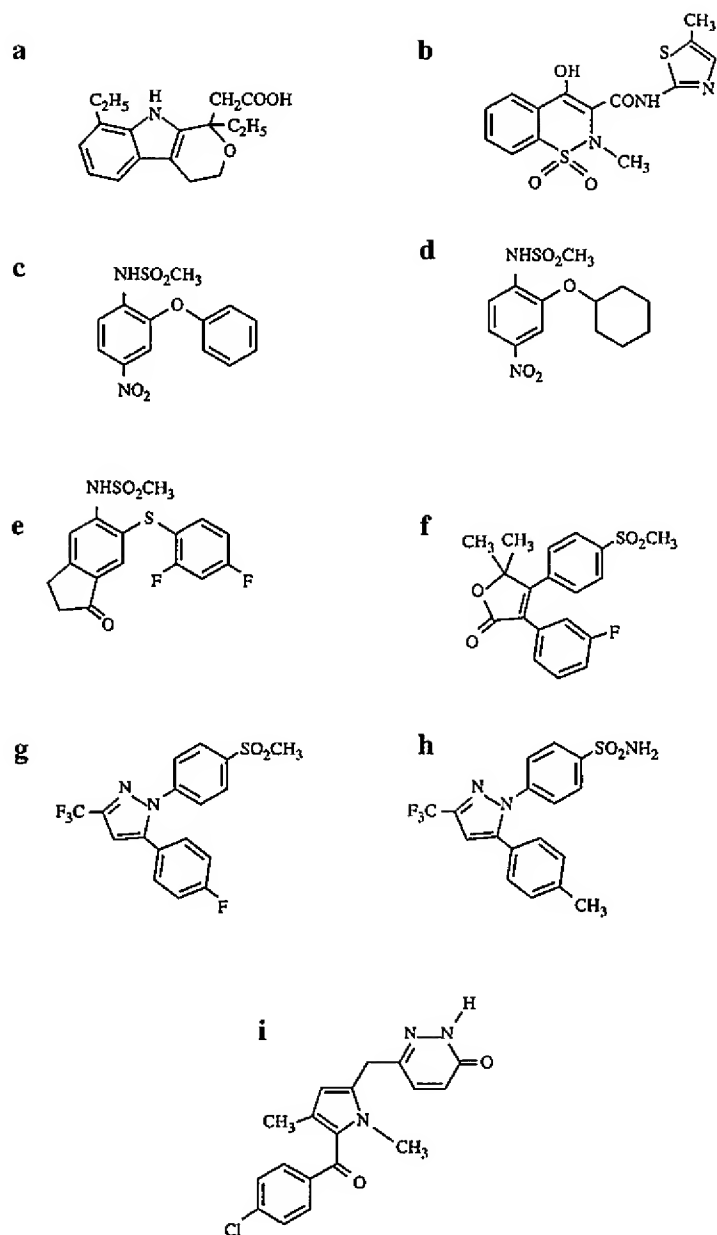


Figure 2 Chemical structures of some selective COX-2 inhibitors. (a) Etodolac; (b) Meloxicam; (c) Nimesulide; (d) NS398; (e) L-745,337; (f) DFU; (g) SC58125; (h) Celecoxib; (i) RS57067000.

Table 1 Comparison of nonsteroid anti-inflammatory drugs for their selectivity towards COX-1 or COX-2

Drug	IC ₅₀ COX-1 (μ M)	IC ₅₀ COX-2 (μ M)	Ratio IC ₅₀ COX-2/COX-1	System
<u>Nonselective for COX-2</u>				
Piroxicam	0.0005	0.3	600	Cultured animal cells (110)
Aspirin	1.67	278	166	Cultured animal cells (110)
Indomethacin	0.028	1.68	60	Cultured animal cells (110)
Diclofenac	1.57	1.1	0.7	Cultured animal cells (110)
6-MNA ^a	278	187	0.67	Human whole blood (111)
<u>Selective for COX-2</u>				
Etodolac	34	3.4	0.1	Human whole blood (111)
Meloxicam	4.8	0.43	0.09	Human whole blood (111)
Nimesulide	9.2	0.52	0.06	Human whole blood (111)
SC58125	38.7	0.27	0.007	Human whole blood (111)
NS398	16.8	0.10	0.006	Human whole blood (111)
L-745, 337	369	1.5	0.004	Human whole blood (111)
Celecoxib	15	0.04	0.003	Human enzymes (80)
DFU	> 50	0.04	<0.001	Human enzymes (81)

^a6-MNA, 6-methoxy-2-naphthyl acetic acid, the active metabolite of nabumetone.

with several 100-fold selectivity for COX-2 (Table 1). The constant molecular motifs in this range of compounds are the absence of a carboxylic group and the presence of a sulphonamide or sulphone moiety (Figure 2). Extensive structure-activity analyses of selective COX-2 inhibitors have been undertaken by the pharmaceutical industry, and some of these have already been reviewed (15).

Table 2 Inhibition of COX-1 and COX-2 by NSAIDs in different systems

System	COX-2/COX-1 Ratio			
	Indomethacin	Nimesulide	Etodolac	Meloxicam
Cultured animal cells	22 (112)	0.05 (122)		0.33 (113)
	60 (110)	0.1 (123)		0.8 (110)
	30 (113)			
	6 (114)			
Human recombinant enzymes	1.3 (115)	0.2 (118)	~0.001 (81)	0.003 (81)
	> 75 (116)	0.02 (124)	0.09 (127)	0.01 (118)
	2.3 (14)	0.16 (125)	0.8 (116)	
	9 (117)	0.01 (81)		
	3.5 (118)	0.02 (120)		
Human whole blood cells	0.51 (119)	0.19 (120)	0.1 (127)	0.09 (111)
	12.5 (120)	0.06 (111)		
	2.9 (121)	0.07 (126)		

However, now that the binding sites for the selective inhibitors in COX-2 have been described in detail and the three-dimensional structure of the enzyme protein clearly established, modern molecular modeling techniques should be able to design de novo compounds binding with high affinity but without the sulphonamide or sulphone group. The compound RS57067000 (Figure 2) may be the first of this class of selective COX-2 inhibitor (9).

Anti-Inflammatory Drugs in Clinical Development

Both celecoxib (80) and MK-966, derived from 5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulphonyl)phenyl-2(5H-furanone) (DFU) (see Figure 2) (81) are in Phase III clinical trials for rheumatoid and osteo-arthritis. So far, both compounds are reported to be effective against inflammation and to cause no gastrointestinal or renal problems. For instance, in healthy volunteers, MK-966 administered daily at 250 mg for 7 days (which is 10 times the anti-inflammatory dose) produced no evidence of gastric damage, as determined by endoscopic examination (82). Celecoxib given for 7 days to volunteers also provided no evidence of gastric damage (83). At 1 g orally, MK-966 had no influence on ex vivo platelet aggregation. Both celecoxib and MK-966 were effective analgesics in humans for moderate to severe pain following dental surgery (84, 85). In animal studies, selective COX-2 inhibitors showed good antipyretic activity within the anti-inflammatory dose range (81, 86). These selective inhibitors and others still under development are also likely to be tested clinically in two other conditions: colon cancer and Alzheimer's disease.

It is clear that much of the pharmacological profile predicted for the highly selective COX-2 inhibitors has been realized, with little or none of the side effects associated with inhibition of COX-1. However, these compounds have been used in a relatively small number of carefully selected patients with high levels of monitoring, as is inevitable in the early stages in their development. The selective inhibitors are aimed at a mass market—approximately 15×10^{12} tablets of aspirin are consumed annually worldwide—and side effects or toxicities of these new compounds will take time and much patient exposure to emerge.

Nevertheless two possible side effects have already been identified, each with serious implications. One concerns wound healing, expressed primarily as the healing of gastrointestinal lesions induced by NSAIDs or other causes. The relevance of COX-2 to wound healing and the associated angiogenesis could reflect the activation of inflammatory cells such as macrophages and the secretion of growth factors such as TGF- β , both conditions known to favor COX-2 induction (6, 87). In rats, COX-2 mRNA and enzyme protein were present in gastric lesions induced by alcohol or acetic acid. Treatment with NS-398, a

selective COX-2 inhibitor, during the acute stage of the damage-delayed healing, although the same treatment in normal rats did not by itself induce ulcers (88). The use of NSAIDs in postoperative analgesia is growing and this would be an obvious extension of use for COX-2 inhibitors (84, 85). Any indication that surgical wound healing might be hindered by the postoperative analgesic would be a serious disincentive to its use.

The other possible side effect is based on the results from the COX-2 knockout mice (44, 45). Particularly, no assessments in animals or in humans of possible effects of COX-2 inhibitors on fetal development or on female fertility have yet been published. Although it could be argued that most female patients with rheumatoid arthritis are postmenopausal and thus at no real risk, some evaluation of potential risk in other possible uses such as postoperative analgesia must be made. Such questions become even more important if the COX-2 inhibitors are to be used prophylactically in the two other conditions, colon cancer and Alzheimer's disease, discussed below.

FUTURE THERAPEUTIC APPLICATIONS FOR COX-2 INHIBITORS

Cancer

Colorectal cancer is a major form of cancer in the Western world; for instance, in the United States it is the next most important cause of cancer deaths after lung cancer. It has been estimated that 50% of those over 70 years old have colorectal adenomata and about 10% of those will progress to cancer (89). The initial evidence for the involvement of COX in colorectal cancer was epidemiological; from more than 10 studies since 1988, a negative correlation emerged between the chronic ingestion of NSAIDs and incidence of colorectal cancer (90–92). In young patients with familial adenomatous polyposis (FAP), a condition in which many colorectal polyps develop spontaneously with eventual progression to tumors, a small trial of sulindac (a nonselective NSAID) showed a significant decrease in number and size of polyps during treatment (93). These indications that COX activity was somehow involved in the progress leading to colorectal cancer were given a crucial scientific basis by the demonstration that COX-2 and not COX-1 was increased in samples of either malignant tissue from colorectal cancer or from polyp tissue from patients (94). A mutant *Apc* mouse is accepted as a model of FAP in humans in which comparable intestinal polyposis develops spontaneously. The number of polyps in these mice was strongly reduced either by treatment with a selective COX-2 inhibitor or by the deletion of the COX-2 gene (95).

In cultures of rat epithelial cells, transfection with COX-2 cDNA and the consequently increased PG synthesis decreased the apoptotic rate. This rate was restored to normal by inhibition of PG production by sulindac (76). Essentially similar results were obtained in vivo with chemically induced colon cancers in rats (96). A diet containing sulindac halved the incidence of adenomacarcinoma and doubled the apoptotic index in the tumor tissue. In an extension of the earlier work in FAP, sulindac caused regression of sporadic adenomatous polyps in 11 out of 15 patients and in 13 out of 20 polyps (97). This is an encouraging result, as most colorectal cancers are sporadic rather than hereditary in origin, but further and larger studies are clearly needed to establish a clinical case for therapy of colorectal adenomatous polyps with COX-2 inhibitors.

The progress from the deletion or mutation of the *Apc* gene, the initial step in the development of colorectal cancer, to the final malignant phenotype comprises at least seven genetic events (89). Clearly, the induction, expression, and activity of COX-2 is an essential step subsequent to loss of the *Apc* protein (75). Loss of *Apc* protein should decrease apoptosis, as the PGs produced by COX-2 appeared to do in cell lines and in vivo (76,96). It may be that the survival of epithelial cells beyond normal lifetimes allows the malignant phenotype to develop.

Whatever the final mechanism, the hitherto unlikely proposition that "aspirin prevents cancer" is now seen to have a foundation in experimental fact at least for one type of tumor. Furthermore, it raises the strong possibility that COX-2 inhibitors could be used to decrease the incidence of colorectal cancer in genetically susceptible subjects, without causing gastrointestinal damage of their own. If this were successful, the prophylactic treatment could be extended to others on the basis of age alone, rather like the presently established aspirin prophylaxis against thromboembolic disease. This lateral development of COX-2 has provided new hope for prevention and perhaps even treatment of colorectal cancer and a new therapeutic use for COX-2 inhibitors, which is being pursued actively by the pharmaceutical industry (98).

Human gastric and breast tumors also express higher levels of COX-2 protein than surrounding normal tissues (29,99). Piroxicam suppressed the growth of human cultured breast cancer cells (100), while sulindac sulfide reduced cancer incidence and the number of cancers per rat in experimental mammary carcinoma induced with 1-methyl-1-nitrosourea (101). Thus, gastric and breast tumors in humans may also be susceptible to treatment with selective COX-2 inhibitors.

Alzheimer's Disease

The correlations between COX, PGs, and Alzheimer's disease were, as for colorectal cancer, initially epidemiological. Several case-control studies between 1988 and 1995 disclosed a significantly reduced odds ratio to almost half of

the normal risk for Alzheimer's disease in those taking NSAIDs as anti-inflammatory therapy (102–104). A report published in April 1997 confirmed the previous findings of an inverse correlation between the severity or incidence of Alzheimer's disease and the ingestion of NSAIDs, with ibuprofen as the most frequently used compound, probably reflecting its availability without prescription (105). In this study, paracetamol use was separated from that of the NSAIDs and was shown to be without benefit.

In all these analyses, the mechanisms proposed are essentially anti-inflammatory and reflect the recognition of inflammatory events and components in the Alzheimer's disease lesions (102, 103, 106). At the site of the plaques, along with the β -amyloid protein, there are activated microglia, complement fragments, release of cytokines, and other classical signs of inflammation. A crucial finding is that β -amyloid is capable of activating microglia. Although the NSAID would not be expected to modify the abnormal metabolism of β -amyloid, they could reduce the response of microglia to the protein. The neuronal damage in Alzheimer's disease may be due more to the inflammatory reaction with the consequent free radical and protease release than to the presence of amyloid per se. Thus, inhibition of inflammation may delay or even abort the loss of neurones consequent on amyloid deposition.

Rat microglia stimulated with LPS express COX-2 (107), and human microglia may respond similarly. With this additional source of COX-2, it was surprising that the total COX-2 content of brain tissue from Alzheimer's disease patients was in fact lower than normal (108). One explanation of this is that in these samples, necessarily from late-stage disease, the loss of neurones and their COX-2 outweighed the increased COX-2 in activated microglia. There may be a detectable increase in the total COX-2 content earlier in the disease process. The lack of a good animal model for Alzheimer's disease has undoubtedly delayed analysis of its causes.

A major benefit of the new selective COX-2 inhibitors could be early treatment in asymptomatic, but genetically at risk, subjects, which could result in a delaying or even preventing the clinical disease. Such treatment with the existing NSAIDs with their propensity to cause gastric damage and platelet malfunction has already been shown to have low compliance (109) and would always be difficult to justify in the asymptomatic subjects targeted. Selective COX-2 inhibitors should, however, enable this prophylactic action of decreased PG synthesis to be fully realized with a minimum of side effects.

CONCLUSIONS

The years since the identification of COX-2 have been exciting and intriguing as well as frustrating and disappointing—in short, typical of scientific progress immediately after a breakthrough. The powerful techniques of molecular biology

have rapidly provided (a) detailed knowledge of the COX-1 and COX-2 proteins, from their linear sequence to their three-dimensional structure, and (b) an extensive description of the gene, its possible transcription factors, and its mRNA. The gene disruption techniques have yielded evidence for the unexpected involvement of either isoform in physiological processes, as well as evidence conflicting with that from pharmacological observations of the effects of COX inhibition.

Although the first generation of selective inhibitors (meloxicam, nimesulide, and etodolac) were discovered by animal screening, the next generation will surely owe more to techniques based on molecular modeling within the active site of the COX proteins. The therapeutic potential of COX-2 inhibition will ensure the continuing development of the basic biology of these proteins, of better inhibitors, and of more effective clinical applications. The beginning of the next millenium may indeed see that not only does aspirin cure cancer but that its siblings will prevent Alzheimer's disease and modify reproductive fertility with a minimum of side effects. Whatever the outcome, there are still many important and interesting questions to be asked and answers to be given about the two (and perhaps more) isoforms of COX.

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Exhibit 12

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Aspirin

Eric H. Awtry and Joseph Loscalzo

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Cardiovascular Drugs

Aspirin

Eric H. Awtry, MD; Joseph Loscalzo, MD, PhD

Salicylates, in the form of willow bark, were used as an analgesic during the time of Hippocrates, and their antipyretic effects have been recognized for more than 200 years.¹ Acetylsalicylic acid, or aspirin, was introduced in the late 1890s² and has been used to treat a variety of inflammatory conditions; however, the antiplatelet activity of this agent was not recognized until almost 70 years later.³ Recent advances in our understanding of the central role of platelets in the pathophysiology of cardiovascular disease have spurred in-depth investigations into the mechanisms of action of aspirin and the clinical utility of this agent in the treatment of common cardiovascular disorders.

Mechanism of Action

Aspirin exerts its effect primarily by interfering with the biosynthesis of cyclic prostanoids, ie, thromboxane A₂ (TXA₂), prostacyclin, and other prostaglandins. These prostanoids are generated by the enzymatically catalyzed oxidation of arachidonic acid, which is itself derived from membrane phospholipids⁴ (Figure). Arachidonic acid is metabolized by the enzyme prostaglandin (PG) H-synthase, which, through its cyclooxygenase (COX) and peroxidase activities, results in the production of PGG₂ and PGH₂, respectively. PGH₂ is then modified by specific synthases, thus producing prostaglandins D₂, E₂, F_{2α}, I₂ (prostacyclin), and TXA₂, all of which mediate specific cellular functions.

PGH-synthase, also referred to as COX, exists in 2 isoforms that have significant homology of their amino acid sequences.⁵ A single amino acid substitution in the catalytic site of the enzyme confers selectivity to inhibitors of the COX isoforms.^{6,7} The first isoform (COX-1) is constitutively expressed in the endoplasmic reticulum of most cells (including platelets)⁸ and results in the synthesis of homeostatic prostaglandins responsible for normal cellular functions, including gastric mucosal protection, maintenance of renal blood flow, and regulation of platelet activation and aggregation.⁴ The second isoform (COX-2) is not routinely present in most mammalian cells but, rather, is rapidly inducible by inflammatory stimuli and growth factors and results in the production of prostaglandins that contribute to the inflammatory response.^{9,10}

Aspirin imparts its primary antithrombotic effects through the inhibition of PGH-synthase/COX by the irreversible acetylation of a specific serine moiety (serine 530 of COX-1

and serine 516 of COX-2)^{11,12} and is ≈170-fold more potent in inhibiting COX-1 than COX-2.¹³ In the presence of aspirin, COX-1 is completely inactivated, whereas COX-2 converts arachidonic acid not to PGH₂, but to 15-*R*-hydroxyeicosatetraenoic acid (15-*R*-HETE).¹⁴ The end result is that neither affected isoform is capable of converting arachidonic acid to PGH₂, a necessary step in the production of prostanoids. The resultant decreased production of prostaglandins and TXA₂ likely accounts for the therapeutic effects, as well as the toxicities, of aspirin. From a cardiovascular standpoint, it is principally the antithrombotic effect of aspirin that results in its clinical utility. Platelet production of TXA₂ in response to a variety of stimuli (including collagen, thrombin, and ADP) results in the amplification of the platelet aggregation response and in vasoconstriction.^{15,16} Conversely, vascular endothelial cell production of prostacyclin results in inhibition of platelet aggregation and induces vasodilation. Aspirin-induced inhibition of TXA₂ and PGI₂ has opposing effects on hemostasis; however, the available data suggest that the potentially prothrombotic effects of PGI₂ inhibition are not clinically relevant and that the antithrombotic effects of TXA₂ inhibition predominate.¹⁷ This may, in part, be a result of the ability of vascular endothelial cells to regenerate new COX and thus recover normal function,¹⁸ whereas COX inhibition in platelets is irreversible owing to the limited mRNA pool and protein synthesis in these anuclear cells.

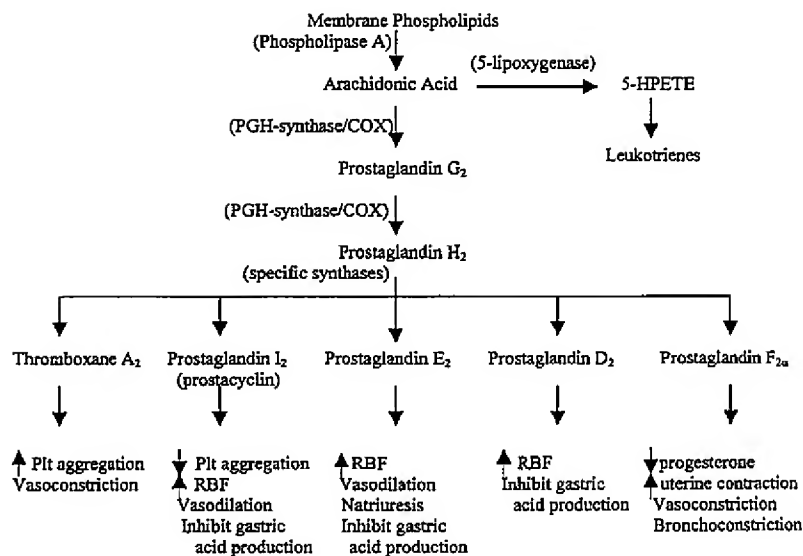
Other mechanisms for platelet inhibition by aspirin have been proposed. For example, aspirin facilitates the inhibition of platelet activation by neutrophils, an effect that appears to be mediated by a nitric oxide (NO)/cGMP-dependent process,¹⁹ and inhibition of prostacyclin synthesis in endothelial cells enhances NO production.²⁰ In addition to its antithrombotic effects, other mechanisms may contribute to the clinical benefits of aspirin in the treatment of cardiovascular disorders. Aspirin may help to decrease the progression of atherosclerosis by protecting LDL from oxidative modification²¹ and also improves endothelial dysfunction in atherosclerotic vessels.²² Several mechanisms have been proposed to explain these benefits, all of which center on the potential role of aspirin as an antioxidant. Salicylate has been shown to be an inhibitor of the cytokine-dependent induction of NOS-II gene expression,^{23,24} perhaps through a mechanism involving nuclear factor-κB activation, an effect that would tend to decrease the nitrosative stress that accompanies cytokine

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The production of prostaglandins from arachidonic acid and their physiological effects. HPETE indicates hydroperoxyeicosatetraenoic acid; PG, prostaglandin; Plt, platelet; and RBF, renal blood flow.

elaboration. Aspirin can also directly scavenge hydroxyl radicals to form the 2,3- and 2,5-dihydroxybenzoate derivatives, which themselves serve as markers of oxidative stress²⁵ and quench oxy-radical flux,²⁶ and can acetylate the ϵ -amino groups of lysine residues in proteins,²⁷ which prevents their oxidation.²⁸ This antioxidant effect on proteins may be important in limiting both lipoprotein oxidation and fibrinogen oxidation; in the latter case, oxidation enhances fibrin formation,^{27,29} and lysine acetylation enhances fibrinolysis.³⁰ It is likely through this combination of effects that aspirin reduces the inflammatory response in patients with coronary artery disease.³¹

Pharmacology/Pharmacokinetics

Aspirin is rapidly absorbed in the upper gastrointestinal (GI) tract and results in a measurable inhibition of platelet function within 60 minutes.^{17,32} This antiplatelet effect is associated with prolongation of the bleeding time and inhibition of TXA₂-dependent platelet aggregation.³³ These effects occur even before acetylsalicylic acid is detectable in the peripheral blood, owing to the exposure of platelets to aspirin in the portal circulation.³⁴ Enteric coating of aspirin significantly delays its absorption.³⁵ The plasma half-life of aspirin is only 20 minutes; however, because platelets cannot generate new COX, the effects of aspirin last for the duration of the life of the platelet (≈ 10 days). After a single dose of aspirin, platelet COX activity recovers by $\approx 10\%$ per day as a function of platelet turnover.³⁶ Although it may take 10 days for the total platelet population to be renewed, and thus restore normal COX activity, it has been shown that if as little as 20% of platelets have normal COX activity, hemostasis may be normal.^{37,38}

The dose of aspirin required to obtain adequate platelet inhibition has been studied extensively. A single dose of 100 mg of aspirin effectively abolishes the production of TXA₂ in normal individuals, as well as in patients with atherosclerotic disease.^{39,40} Single doses below 100 mg result in a dose-dependent effect on TXA₂ production; the effect of repeated

daily doses is cumulative, although >24 hours may be required to achieve maximal COX inhibition.^{38,39,41} Therapeutic benefit in a variety of cardiovascular diseases has been demonstrated with doses of 30 to 1500 mg/d; higher doses do not appear to be more effective but may increase the risk of GI side effects.^{17,42} Low-dose aspirin or controlled-release preparations may result in somewhat preferential inhibition of platelet COX over endothelial COX.^{33,40,43} This differential effect has theoretical advantages in that intact endothelial PGI₂ production may enhance the antithrombotic effects of aspirin; however, the clinical importance of maintaining normal PGI₂ production remains undetermined.

Aspirin in Coronary Artery Disease

Acute Therapy

Acute Myocardial Infarction

The importance of platelets and thrombosis in the pathophysiology of acute coronary syndromes is well established. Although early studies of the use of aspirin as an antithrombotic agent in the acute treatment of myocardial infarction (MI) yielded conflicting results, the Second International Study of Infarct Survival (ISIS-2)⁴⁴ has since unequivocally established the benefit of aspirin in this setting. In this trial, 17 187 patients presenting within 24 hours of the onset of a suspected acute MI (AMI) were randomized to receive intravenous streptokinase (1.5 MU), 162.5 mg of aspirin daily for 30 days, both, or neither. At the end of 5 weeks, patients receiving aspirin therapy alone had a highly significant 23% reduction in vascular mortality and a nearly 50% reduction in the risk of nonfatal reinfarction and nonfatal stroke. This benefit occurred irrespective of whether heparin was given. These reductions translate into the avoidance of ≈ 25 deaths and 10 to 15 nonfatal reinfarctions or strokes by treating 1000 patients with aspirin for 1 month. Additionally, there was no increase in major bleeding complications (including no increase in cerebral hemorrhage or need for transfusion) with

TABLE 1. Benefit of Aspirin in Unstable Angina

Reference	No. of Patients	Dose, mg	Duration of Treatment	% Death or Nonfatal MI			% Mortality		
				Aspirin	Control	% Reduction (P)	Aspirin	Control	% Reduction (P)
VA Cooperative Study ⁵³	1266	325 QD	12 wk	5.0	10.1	51 (0.0005)	1.6	3.3	51 (0.054)
Canadian Multicenter Trial ⁵⁴	555	325 QID	24 mo	8.6	17.0	51 (0.008)	3.0	11.7	71 (0.004)
Thérault et al ⁵⁵	479	325 BID	6 d	3.3	12.0	72 (0.01)	0.0	1.7	...
RISC ^{52,56,57*}	796	75 QD	5 d	2.5	5.8	57 (0.033)	0.25	0.25	0 (NS)
			6 mo	8.9	19.0	53 (<0.0001)	2.0	3.8	47 (NS)
			12 mo	11.0	21.4	49 (0.0001)	2.8	4.5	38 (NS)

*Included patients with unstable angina and non-Q-wave infarctions. Results were similar in both groups.

aspirin therapy, and the mortality benefit was maintained after 10 years of follow-up.⁴⁵

In the past decade, thrombolytic therapy has become the cornerstone of medical management of AMI.^{46,47} Aspirin, however, remains an important adjunctive therapy. In ISIS-2,⁴⁴ administration of streptokinase alone was associated with a 25% reduction in vascular deaths, and the effect of aspirin therapy was additive (42% reduction in vascular mortality with combined aspirin and streptokinase therapy). Additionally, an excess of nonfatal reinfarctions was seen in the first several days after treatment with streptokinase alone, likely as a result of plasmin-induced platelet activation; this increase was entirely prevented by the concomitant use of aspirin. Compared with aspirin as an adjunct to thrombolysis, heparin appears to be associated with a higher early patency rate of the infarct-related artery, although aspirin was associated with a trend toward a decreased 7-day reocclusion rate.⁴⁸ The addition of heparin to aspirin does not clearly decrease mortality or reinfarction and is associated with an increase in bleeding complications.^{49,50} A meta-analysis of 32 trials using aspirin as adjunctive therapy to thrombolysis demonstrated significantly decreased reocclusion rates (11% versus 25%) and recurrent ischemic events (25% versus 41%) with aspirin therapy.⁵¹

Unstable Angina and Acute Non-ST-Segment-Elevation MI

Several studies have clearly demonstrated a beneficial role for aspirin in the treatment of unstable angina (Table 1).^{52–55} Despite instituting aspirin therapy at various doses (75 to 1300 mg/d) and differing intervals after a patient's initial presentation (<24 hours to <8 days), these trials have consistently demonstrated a significant decrease in the incidence of death or death and nonfatal MI. Additionally, in the Research Group on Instability in Coronary Artery Disease in Southeast Sweden (RISC) trial,⁵⁶ treatment with aspirin (75 mg/d) decreased the progression to severe angina necessitating cardiac catheterization by 40% at 3 months (10.8% versus 18.1%) and 29% at 12 months (20.8% versus 29.2%). Low-dose aspirin (75 mg/d) has also been shown to decrease the risk of MI or death in patients with asymptomatic ischemia on treadmill testing after an episode of unstable angina or a non-Q-wave MI.⁵⁷ A review of ≈4000 patients with unstable angina treated with aspirin or placebo demonstrated a 5% absolute risk reduction in nonfatal stroke or MI

or vascular death (9% versus 14%)⁴²; this corresponds to 50 vascular events avoided per 1000 patients treated with aspirin for 6 months.

Aspirin has been compared with heparin as both alternative and adjunctive therapy in the setting of unstable angina. In the RISC trial,⁵⁶ treatment with heparin alone provided no significant benefit for the incidence of MI and death, although the significant delay in instituting heparin therapy likely contributed to this finding (average delay 33 hours). Aspirin therapy was significantly better than heparin; however, the combination of aspirin and heparin produced the greatest benefit. Other studies have demonstrated a greater benefit of heparin over aspirin therapy^{55,58,59} and a potential increase in bleeding complications with combination therapy.⁵⁵ In a recent meta-analysis, the addition of heparin to aspirin therapy in unstable angina and non-Q-wave MI resulted in a nonsignificant 33% decrease in the risk of MI or death compared with aspirin alone; this benefit occurred without an increase in bleeding complications.⁶⁰ In addition, therapy with aspirin may prevent the early reactivation of angina observed after discontinuation of heparin therapy.⁶¹

Secondary Prevention

After MI

There have been 6 large, randomized trials that used aspirin alone as long-term treatment after an AMI,^{62–67} and all but 1 of these⁶² demonstrated a trend toward decreased mortality with aspirin therapy. The results of these trials and 139 others that evaluated the long-term use of aspirin in a wide range of patients were reviewed in a meta-analysis by the Antiplatelet Trialists in 1994.⁴² This analysis comprised ≈100 000 patients, 70 000 of whom were considered "high-risk patients" by virtue of a prior history of AMI, unstable angina, stable angina, prior percutaneous or surgical coronary revascularization, stroke, transient ischemic attack (TIA), atrial fibrillation, valvular heart disease, or peripheral vascular disease. Overall, among these high-risk patients, aspirin reduced the risk of nonfatal MI by approximately one-third, the risk of nonfatal stroke by one-third, and the risk of vascular death by one-sixth.

Among ≈20 000 of these patients with a prior history of MI, aspirin therapy decreased the risk of vascular events over an average 2-year treatment period from 17.1% to 13.5%, corresponding to an absolute decrease of 36 events per 1000 patients treated. Among 11 000 patients with a prior stroke or

TABLE 2. Studies of Aspirin for Primary Prevention of Cardiovascular Events

Reference	No. of Patients	Dose, mg	Length of Follow-up	Total Mortality, RR (95% CI)	Nonfatal MI, RR (95% CI)	Stroke, RR (95% CI)
Physicians' Health Study ⁸¹	22 071	325	5 y	0.96 (0.80–1.14)	0.59 (0.47–0.74)	1.22 (0.93–1.60)
Peto et al ⁸²	5139	500	6 y	0.89*	0.97*	1.15 (0.75–1.50)
Thrombosis Prevention Trial ⁸⁴	5085	75	6.4 y	1.06 (0.88–1.28)	0.68 (0.52–0.88)	0.98 (0.65–1.45)
Manson et al† ⁸⁵	87 678	Varied	5.4 y	0.86 (0.72–1.03)	0.68 (0.49–0.93)	0.99 (0.71–1.36)

RR is for aspirin vs nonaspirin groups.

*Result not statistically significant; CIs not available.

†RRs for subgroup taking up to 6 aspirin per week vs nonaspirin group.

TIA, aspirin therapy was associated with an event rate of 18.4% compared with a rate of 22.2% in control subjects (3-year decrease in absolute event rate of 38 events per 1000 patients). In other high-risk patients, the benefit was somewhat less but still significant: the 1-year benefit in this group was ≈20 events per 1000 patients treated with aspirin.

These results clearly demonstrate a significant treatment effect of aspirin when given as secondary prevention in patients with underlying cardiovascular disease. Additionally, the results were significant in all groups irrespective of age, gender, or the presence of hypertension or diabetes. A wide range of dosing regimens was evaluated in this trial (most frequently 75 to 325 mg/d), and these regimens were equally effective. Given the effectiveness of a dose of 162.5 mg/d in the ISIS-2 trial⁴⁴ and the higher incidence of GI side effects when aspirin is used chronically at higher doses (see below), it seems reasonable to begin treatment with a dose of 160 to 325 mg and continue chronic treatment with 75 to 160 mg/d in patients with coronary artery disease.

After Revascularization

Percutaneous revascularization with balloon angioplasty or intracoronary stenting results in local vascular trauma, with exposure of the subendothelium to the vascular space. This highly thrombogenic milieu predisposes to intraluminal thrombus development with either abrupt closure or subacute thrombosis of the vessel in 3.5% to 8.6% of procedures.^{68–70} Several studies have demonstrated a significant decrease in acute complications of angioplasty with the combination of aspirin and dipyridamole,⁷¹ although this combination provides little additional benefit over aspirin alone.⁷² Compared with aspirin alone or a regimen of aspirin plus warfarin, the combination of ticlopidine (500 mg/d for 1 month) and aspirin (325 mg/d) in patients undergoing intracoronary stent placement significantly decreases the 30-day combined end point of death, target-vessel revascularization, angiographic thrombosis, or MI (relative risk [RR] 0.15 for combined therapy versus aspirin alone).⁷³ This benefit is seen irrespective of whether the stent deployment is felt to be “successful” with a low risk for thrombosis⁷³ or if high-risk markers for stent thrombosis are present.⁷⁴

Coronary artery bypass surgery with saphenous vein grafts is associated with a 5% to 15% graft occlusion rate during the first postoperative month,^{75,76} which is largely related to thrombosis at the anastomotic site as a result of endothelial disruption and vessel damage.⁷⁷ When given in the immediate postoperative period, aspirin clearly decreases the rate of

early thrombotic graft occlusion by ≈50%, and continued aspirin therapy for 1 year further decreases the rate of occlusive events.^{75,76} Preoperative administration of aspirin is associated with increased bleeding complications but offers no additional benefit in early graft patency compared with providing aspirin 6 hours after surgery.⁷⁸ Although there does not appear to be additional benefit of aspirin with regard to long-term graft patency after 1 year of therapy,⁷⁹ continued aspirin therapy is required for secondary prevention of vascular events in these patients. Treatment with ticlopidine or sulfinpyrazone also improves early graft patency; however, these agents have not been shown to be better than aspirin.⁸⁰

Primary Prevention

In light of the benefit of aspirin in the treatment of acute cardiovascular disease and in the secondary prevention of recurrent events, enthusiasm has developed for the evaluation of aspirin as a primary preventive measure (Table 2). There have been 2 large, randomized trials of aspirin for the primary prevention of cardiovascular events that enrolled male physicians without prior MI and with a low incidence of prior cardiovascular disease (eg, TIA or angina).^{81,82} The Physicians' Health Study randomized 22 071 subjects between the ages of 40 and 84 years to treatment with aspirin (325 mg every other day) or placebo.⁸¹ The study was stopped prematurely after an average follow-up of 5 years owing to a highly significant 44% reduction in the risk of MI in the aspirin-treated group (0.26% per year versus 0.44% per year), an effect that was limited to participants over the age of 50 years. Nonetheless, there was no decrease in cardiovascular mortality. Additionally, there was a nonsignificant increase in hemorrhagic stroke (RR 2.14) and a significant increase in GI bleeding requiring transfusion. The British Physicians' Study enrolled 5139 subjects and also demonstrated no difference in cardiovascular mortality after 6 years of aspirin therapy (500 mg/d).⁸² Importantly, this trial showed no significant difference in the incidence of MI but a significant increase in disabling strokes. Combined analyses of these results demonstrated a significant 33% treatment-related reduction in nonfatal MI but still failed to show a decrease in mortality and demonstrated a borderline increase in hemorrhagic strokes and a nonsignificant increase in all strokes.^{42,83}

These 2 trials studied a population of patients who have a very low risk for cardiovascular events. Individuals at higher risk for the development of cardiovascular events (based on their risk factor profile) were enrolled in the Thrombosis Prevention Trial⁸⁴ and randomized to aspirin (75 mg/d),

warfarin (average dose 4.1 mg/d), both, or neither. After >6 years of follow-up, there was a 20% reduction in ischemic heart disease events (cardiac death, fatal or nonfatal MI) in the aspirin-treated groups. This difference was almost entirely accounted for by a 32% reduction in nonfatal events, without a significant effect on mortality. In contrast, warfarin therapy resulted in a 21% reduction in ischemic events, mostly as a result of a 39% reduction in fatal events. Neither of these therapies alone resulted in an increase in the total number of strokes. The combination of aspirin and warfarin produced the greatest reduction in ischemic events (34%) but was also associated with an increase in hemorrhagic and fatal strokes.

Patients with chronic stable angina have a significant risk of developing subsequent cardiovascular events,⁸⁵ and several studies have demonstrated a beneficial effect of aspirin in this group of patients. In the Physician's Health Study, patients who had chronic stable angina and received aspirin had an 87% reduction in the risk of MI compared with their counterparts who received placebo.⁸⁶ Similarly, in the Swedish Angina Pectoris Aspirin Trial, 2035 patients with chronic stable angina but without prior MI who received aspirin (75 mg/d) had a 34% decrease in the combined risk of MI and sudden death.⁸⁷ The risk of stroke, however, was increased by aspirin use in both studies.

No randomized data are available regarding the use of aspirin for the primary prevention of cardiovascular disease in women. However, in a prospective cohort study of 87 678 US nurses, the use of up to 6 aspirin per week did not alter the risk of cardiovascular death, stroke, or important vascular events.⁸⁸ The risk of first MI was significantly reduced (RR 0.68), although this beneficial effect was limited to women over the age of 50 years. These findings are consistent with the results of primary prevention trials in men; however, definitive recommendations await the results of the ongoing Women's Health Study.⁸⁹

In summary, the primary prevention trials demonstrate that aspirin therapy does not decrease cardiovascular mortality but significantly decreases the risk of nonfatal MI. There does not appear to be a consistent effect on the incidence of stroke, although there is a trend toward an increase in stroke risk. Additionally, there is an increase in nonfatal bleeding. The absolute benefit of aspirin therapy clearly increases as the risk of cardiovascular events increases in the treatment group (Table 3). Therefore, in patients with a relatively low risk of developing cardiovascular disease, the risk of prophylactic aspirin therapy may be outweighed by the risk of hemorrhagic complications. Conversely, in patients believed to be at high risk, the benefits of therapy, specifically a decrease in the development of MI, may outweigh the risk of hemorrhagic complications, and prophylactic therapy may be warranted.

Aspirin in Cerebrovascular Disease

Acute Therapy

Two large, randomized trials of aspirin use in the setting of an acute, ischemic stroke have recently been reported (Table 4).^{90,91} Combined, these trials enrolled >40 000 patients within 48 hours of the onset of neurological symptoms and

TABLE 3. Gradient of Benefit in Trials of Aspirin Therapy

Indication for Therapy	Magnitude of Benefit
Treatment of AMI	24 deaths/1000 patients treated for 5 wk
Treatment of unstable angina	50 events/1000 patients treated for 6 mo
Secondary prevention	
After MI	36 events/1000 patients treated for 2 y
After CVA/TIA	38 events/1000 patients treated for 2 y
Primary prevention	
In patients with angina	51 events/1000 patients treated for 4 y
In "high-risk" patients	5 events/1000 men treated for 1 y
In "low-risk" patients	4 events/1000 men treated for 5 y

CVA indicates cerebrovascular accident.

"High-risk" refers to patients in the top 20% of risk based on their risk factor profile.⁸⁴ "Low-risk" refers to patients with minimal risk factors for atherosclerotic cardiovascular disease.

demonstrated a significant decrease in the risk of recurrent stroke and in the combined incidence of death or nonfatal stroke (Table 4). Importantly, there was no significant increase in hemorrhagic stroke. These results correspond to a reduction of 10 deaths or recurrent strokes per 1000 patients after 2 to 4 weeks of aspirin therapy. These trials also demonstrated a trend toward a decreased incidence of death or significant disability (dependence) at 4 weeks of follow-up. The addition of heparin (5000 or 12 500 IU subcutaneously, twice a day) to aspirin yielded no further benefit but increased bleeding complications.⁹⁰ In addition, heparin therapy alone effected no difference in the rate of death or recurrent stroke but resulted in a significant increase in hemorrhagic strokes and major noncerebral bleeding.

Secondary and Primary Prevention

There are conflicting results from individual trials regarding the effectiveness of aspirin in the secondary prevention of cerebrovascular events.⁹²⁻⁹⁵ Included in the Antiplatelet Trialists' review were 12 randomized trials of >10 000 patients with a prior stroke or TIA.⁴² Most of these patients were treated with aspirin (50 to 1500 mg/d), although some received other antiplatelet agents, either alone or in combination with aspirin. Overall, there was a highly significant 17% reduction in the risk of nonfatal stroke and of all vascular events (nonfatal stroke or MI or vascular death) in patients treated for a mean of 33 months. This effect was similar whether the patient presented with a TIA or a completed stroke and resulted in a reduction of 37 vascular events per 1000 patients treated. Similar results have been reported in 3 subsequent trials.⁹⁶⁻⁹⁸ In a recent meta-analysis of 10 randomized trials comprising 9172 patients with cerebrovascular disease who were given prolonged aspirin administration, aspirin resulted in a significant 13% reduction in the risk of subsequent stroke compared with placebo.⁹⁹

Overall, data regarding the use of aspirin for the primary prevention of strokes in patients at high risk are not encouraging. In the British Physicians' Study,⁸² aspirin therapy significantly decreased the incidence of TIA (15.9% versus 27.5%; $P<0.05$) but did not decrease the risk of stroke and in fact increased the risk of disabling stroke (19.1% versus

TABLE 4. Trials of Aspirin Therapy in Acute Ischemic Stroke

End Point	Chinese Acute Stroke Trial ⁹¹			International Stroke Trial ⁹⁰		
	Aspirin	No Aspirin	2P	Aspirin	No Aspirin	2P
Death	3.3	3.9	0.04	9.0	9.4	NS
Death and nonfatal CVA	5.3	5.9	0.03	11.3	12.4	<0.05
Recurrent CVA	1.6	2.1	0.01	2.8	3.9	<0.001
Hemorrhagic CVA	1.1	0.9	NS	0.9	0.8	NS
Death or dependence	30.5	31.6	0.08	62.2	63.5	0.07

CVA indicates cerebrovascular accident. Values are percentages (except for 2P values).

In the International Stroke Trial, follow-up was at 2 weeks except for "death or dependence," which was evaluated at 6 months. In the Chinese Acute Stroke Trial, follow-up was at 4 weeks.

7.4%; $P<0.05$). Similarly, an increased risk of stroke, primarily of the hemorrhagic type, was noted in the Physicians' Health Study.⁸¹ In a small study of asymptomatic patients with carotid bruits and $\geq 50\%$ stenosis of a carotid artery, aspirin failed to prevent subsequent cerebrovascular events.¹⁰⁰ Four placebo-controlled trials have evaluated aspirin for the prevention of stroke in patients with atrial fibrillation^{101–104} and, when their data are combined, demonstrate a small but significant reduction in risk.¹⁰⁵ However, except in the very-low-risk patient (age <65 years with no other cardiovascular disease), the reduction in stroke risk is much greater with warfarin therapy in trials that directly compare the 2 agents (68% versus 12%).^{101,103,105–107}

The ideal dose of aspirin for the prevention of future vascular events in patients with TIAs or minor stroke has been the subject of much debate,^{108,109} although several trials have demonstrated increased bleeding complications with higher doses.^{95,98} In the meta-analysis mentioned above, the beneficial effect of aspirin on the incidence of recurrent stroke occurred irrespective of dose (50 to 1500 mg/d).⁹⁹ Additionally, in a large group of patients undergoing carotid endarterectomy, low-dose aspirin (81 or 325 mg/d) was associated with a lower risk of stroke, MI, or death compared with high-dose regimens (650 or 1300 mg/d).^{110,111} Thus, as is the case with coronary artery disease, a low-dose aspirin regimen appears appropriate for secondary prevention of cerebrovascular disease.

Adverse Effects

The inhibition of prostaglandin synthesis is responsible for the anti-inflammatory effects of aspirin but also results in the alteration of normally protective prostaglandin functions with potentially serious consequences, including gastric ulcers,

renal failure, and impaired platelet function with resultant hemorrhagic complications. These side effects and others will be discussed next.

GI Toxicity

Aspirin-induced inhibition of COX results in loss of the cytoprotective effects of PGE₂ on the gastric mucosa. This mechanism likely accounts in part for the more frequent development of GI side effects in the aspirin-treated patients in most trials.^{93,95,97,112} Minor GI symptoms (including nausea, vomiting, heartburn, and indigestion) have been reported in 5.2% to 40% of patients treated with aspirin versus 0.7% to 34% of patients taking placebo,^{52,54,62,81,95} peptic ulcers in 0.8% to 2.6% of aspirin-treated patients versus 0% to 1.2% with placebo,^{81,82,93} and major GI bleeding (melena requiring transfusion or hematemesis) in $<1\%$ of patients in both groups.^{53,81,84,87,90} Minor bleeding episodes (epistaxis, hematuria, melena not requiring therapy, and bruising) occur frequently in patients taking aspirin and are significantly more common than among their placebo-treated counterparts.^{84,104,113} In the United Kingdom Transient Ischaemic Attack (UK-TIA) trial,⁹⁵ the incidence of GI symptoms was not only significantly higher in the aspirin-treated group than in the placebo group, but GI symptoms were significantly more frequent in the high-dose (1200 mg/d) than in the low-dose (300 mg/d) aspirin groups ($2P<0.001$ for both comparisons). An overview of randomized trials of aspirin therapy similarly found that GI toxicity (both major and minor) was dose related with daily doses between 30 and 1300 mg.¹¹² Nonetheless, even low doses of aspirin (50 to 75 mg/d) are not free from side effects, may still be associated with increased GI bleeding,^{97,104,113} and frequently precipitate the discontinuation of therapy.^{52,56}

TABLE 5. Recommendations for Aspirin Use

Clinical Indication	Recommended Dose
For treatment of	Initial therapy: 160–325 mg
AMI	Subsequent daily dose: 75–160 mg
Acute thromboembolic stroke	
Unstable angina	
Secondary prevention after MI, stroke, or TIA and in patients with chronic stable angina	Daily therapy with 160–325 mg
Primary prevention	No clear indication at this time. Consider therapy with 75–160 mg/d in patients believed to be at high risk for development of cardiovascular disease.

Hemorrhagic Stroke

Several studies have suggested an increase in the risk of hemorrhagic stroke in patients treated with aspirin in the setting of an AMI⁴⁴ or acute ischemic stroke,^{90,91} as well as when aspirin is used for the primary⁸¹ or secondary⁹⁷ prevention of cardiovascular events. A recent meta-analysis of 16 trials comprising 55 462 patients treated with aspirin or control therapy demonstrated a significant increase in hemorrhagic strokes (RR 1.84; $P < 0.001$) despite a decrease in ischemic strokes, total strokes, and MI.¹¹⁴ This relative risk translated into an absolute increase of 12 hemorrhagic strokes per 10 000 patients treated with aspirin.

Other Side Effects

The use of nonaspirin inhibitors of COX (nonsteroidal anti-inflammatory drugs [NSAIDs]) may be associated with an increased risk of renal insufficiency and worsening of hypertension control owing to inhibition of renal vasodilatory prostaglandins.^{115,116} Aspirin is a relatively weak inhibitor of renal prostaglandin synthesis and does not significantly affect renal function or blood pressure control when used at the low to moderate doses suggested for the treatment of cardiovascular disease.¹¹⁷ However, at high doses (1500 mg/d), aspirin can significantly reduce renal sodium excretion in patients with heart failure.¹¹⁸ Aspirin has been reported to counteract the systemic arterial vasodilatory effects and attenuate the mortality benefit of ACE inhibition by enalapril in patients with congestive heart failure.^{119–121} A similar loss of efficacy was not seen in a post hoc analysis of the Captopril and Thrombolysis Study.¹²² A recent review of the literature in this regard suggests that low-dose aspirin (≤ 100 mg/d) has very little interaction with the effects of ACE inhibitors, whereas higher doses may attenuate the benefit of these agents in patients with hypertension or congestive heart failure.¹²³

A small percentage of people, most of whom have preexisting asthmatic disease, suffer from aspirin intolerance or sensitivity. Administration of aspirin to these persons results in the development of bronchoconstriction, rhinitis, and/or urticaria.¹²⁴ The mechanism of this sensitivity is not known but likely results from the inhibition of COX and possibly from abnormal leukotriene production.¹²⁵ Aspirin sensitivity can result in severe respiratory decompensation; however, most patients can be safely desensitized by the gradual administration of increasing doses of aspirin.

Making a Safer Aspirin

Attempts have been made to decrease the gastric toxicity of aspirin by pharmacological manipulation. Sustained-release⁴³ and topical formulations¹²⁶ have been demonstrated to produce relatively selective inhibition of platelet TXA₂ production with minimal effects on vascular and gastric prostanoids and thus may have less gastrototoxicity. Enteric-coated aspirin tablets may be less gastrototoxic as a result of decreased gastric irritation. In a small endoscopic study of asymptomatic patients undergoing long-term aspirin therapy,¹²⁷ gastric mucosal erosions were noted in 90% of patients treated with regular aspirin compared with 60% of patients receiving enteric-coated aspirin. Additionally, GI blood loss has been

shown to be less with enteric-coated aspirin than with the noncoated formulation.¹²⁸ Nonetheless, because the mechanism of action of enteric-coated aspirin still leads to the systemic inhibition of COX, coated aspirin is associated with significant gastric toxicity compared with placebo¹²⁷ and results in a similar risk of upper GI bleeding compared with regular, uncoated aspirin.¹²⁹

Regular aspirin is rapidly absorbed from the acid environment of the stomach. Enteric coating of aspirin results in its release into the alkaline environment of the small bowel, where it is hydrolyzed. As a result, enteric-coated aspirin has lower bioavailability than regular aspirin.¹³⁰ Nonetheless, the antiplatelet effects of full-dose (>300 mg) enteric-coated aspirin are similar to those of uncoated formulations.^{130,131} However, the efficacy of low-dose (<100 mg) enteric-coated preparations has not been clearly established, and it is possible that such doses may result in inadequate platelet inhibition. Thus, if coated aspirin is prescribed, larger doses may be necessary to obtain the desired antiplatelet effect.

The dissociation of the effects of the different COX enzymes (COX-1 and COX-2) has stimulated the production of agents that preferentially inhibit COX-2 and allow for the inhibition of inflammatory prostaglandins while leaving homeostatic prostaglandins relatively intact. Several new NSAIDs have been shown to have relative COX-2 selectivity^{132–134} and appear to be associated with fewer gastric side effects.^{7,135,136} The therapeutic antithrombotic effects and the toxic gastric effects of aspirin are both mediated through the inhibition of COX-1; therefore, dissociation of these effects is not feasible. However, coadministration of aspirin with the synthetic PGE₂ analog misoprostol allows for the complete inhibition of TXA₂ synthesis in platelets while maintaining gastric protection. This approach decreases the risk of gastric ulceration, erosion, and hemorrhage in dogs.^{137,138} Furthermore, in a randomized trial in healthy volunteers given anti-inflammatory doses of aspirin (3900 mg/d), cotreatment with 200 mg of misoprostol twice daily significantly reduced endoscopically documented gastric and duodenal mucosal injury ($P < 0.006$).¹³⁹

Other novel methods of improving the safety profile of aspirin are being developed. Animal models suggest that the intragastric administration of aspirin stimulates the release of NO, which decreases gastric acid secretion and increases cytoprotection, thus limiting gastric mucosal damage.¹⁴⁰ Furthermore, compared with regular aspirin, the administration of NO-releasing derivatives of aspirin has no topical gastric irritating effects, does not worsen stress-induced gastric ulceration, and protects against toxic gastric injury.^{141–143} This marked improvement in gastric toxicity occurs with these agents despite the equivalent inhibition of COX and equipotent or enhanced antithrombotic activity compared with aspirin.¹⁴³ The clinical safety and efficacy of these agents remain to be determined.

Comparison With Other Antiplatelet Agents

Despite aspirin's demonstrated effectiveness in treating and preventing atherosclerotic disease, it produces only partial inhibition of platelet aggregation, and therefore it is a relatively weak antiplatelet agent. Additionally, a minority of

patients appear to be relatively resistant to the antiplatelet effects of aspirin, even when it is administered in large doses.¹⁴⁴ Platelet aggregation studies have demonstrated incomplete inhibition of aggregation in 25% of patients with prior ischemic stroke who were receiving long-term aspirin therapy (minimum dose 325 mg/d).¹⁴⁵ Some patients demonstrate improved platelet inhibition at higher aspirin doses; however, 8% of patients taking 1300 mg of aspirin per day may still be aspirin resistant.¹⁴⁵ The mechanism of this decreased efficacy of aspirin in some patients is not well understood but may reflect the limited potency of aspirin as an inhibitor of COX-2, the expression of which has recently been demonstrated in human platelets.¹⁴⁶

Aspirin does not completely inhibit TXA₂ synthesis,⁴¹ and other non-TXA₂-dependent activators of platelet aggregation (eg, thrombin, ADP, and collagen) can bypass the aspirin-inhibitory effect and result in thrombosis. Newer agents that interrupt these other pathways or interfere with the glycoprotein IIb/IIIa receptor, the final common pathway in platelet aggregation, may prove to be more effective antithrombotic agents.¹⁴⁷ Several other antiplatelet agents have therefore been used for the treatment of thrombotic cardiovascular disease and have been compared with aspirin in randomized clinical trials.

In the Antiplatelet Trialists' overview, several antithrombotic regimens were evaluated, including aspirin, ticlopidine, or sulfapyrazone alone or the combination of aspirin plus dipyridamole.⁴² Direct and indirect comparisons of the effectiveness of these regimens demonstrated no significant difference in vascular events, although the numbers of patients enrolled in trials that directly compared agents were low.

Ticlopidine and Clopidogrel

Ticlopidine and clopidogrel are thienopyridine derivatives that inhibit ADP-induced binding of fibrinogen to platelets, a process necessary for platelet aggregation.¹⁴⁸ In randomized trials of patients with recent stroke or TIA, ticlopidine (250 mg twice daily) has demonstrated a significant 23.3% reduction in the combined incidence of stroke, MI, or vascular death compared with placebo (11.3% per year versus 14.8% per year with placebo; $P=0.02$),¹⁴⁹ as well as a 21% lower risk of stroke (10% versus 13%; $P=0.024$) and a 12% reduction in the combined risk of death and nonfatal stroke (17% versus 19%; $P=0.048$) compared with aspirin (650 mg twice daily).¹⁵⁰ However, ticlopidine therapy resulted in severe neutropenia in $\approx 1\%$ of patients.

The Clopidogrel versus Aspirin in Patients at Risk for Ischemic Events (CAPRIE) study compared the efficacy of aspirin (325 mg/d) with clopidogrel (75 mg/d) for reducing the combined incidence of ischemic stroke, MI, or vascular death in 19 185 patients with a recent stroke or MI or with symptomatic peripheral arterial disease.¹⁵¹ After an average follow-up of almost 2 years, clopidogrel demonstrated a significant 8.7% benefit over aspirin (5.32% versus 5.83%; $P=0.043$). Adverse events were not significantly different between the agents, and neutropenia was rare (0.1%) with clopidogrel.

Dipyridamole

Dipyridamole is a pyrimidopyrimidine derivative that inhibits cyclic nucleotide phosphodiesterases and blocks the uptake of adenosine, resulting in a reduction in platelet cytosolic calcium and subsequent inhibition of platelet activation.¹⁵² Initial studies demonstrated no significant benefit of adding dipyridamole to aspirin for the secondary prevention of stroke⁹⁴ or recurrent MI.¹⁵³ The European Stroke Prevention-2 trial randomized 6602 patients with prior minor stroke or TIA to treatment with aspirin (50 mg/d), dipyridamole (400 mg/d), both, or neither. After 2 years of follow-up, the 2 agents alone were found to be equally effective in reducing the risk of stroke (RR reductions: 18% with aspirin, $P=0.013$; 16% with dipyridamole, $P=0.039$) and stroke or death combined (RR reductions: 13% with aspirin, $P=0.016$; 15% with dipyridamole, $P=0.015$) compared with placebo.¹⁰⁴ Furthermore, the benefits were additive with combination therapy (RR reductions: 37% for stroke, $P<0.001$; 24% for combined end point, $P<0.001$). A recent review of 15 randomized trials suggests that the addition of dipyridamole to aspirin will reduce the risk of vascular events by an additional 15% over the effects of aspirin alone.⁹⁹

Glycoprotein IIb/IIIa Inhibitors

Irrespective of the activating stimulus, the final common pathway of platelet activation involves exposure and activation of glycoprotein IIb/IIIa, the platelet fibrinogen receptor. Inhibitors of this receptor, including monoclonal antibodies and peptide- and nonpeptide-derived agents, have been studied extensively in various settings. When added to standard antiplatelet therapy with aspirin (325 mg) and intravenous heparin in patients undergoing percutaneous revascularization, the monoclonal antibody c7E3 (abciximab) reduced the risk of ischemic complications (death, nonfatal MI, unplanned revascularization procedures, or refractory angina) by 35% (8.3% versus 12.8% with placebo; $P=0.008$) in patients undergoing high-risk angioplasty (unstable angina, evolving AMI, or high-risk coronary morphology)¹⁵⁴ and by 56% (5.2% versus 11.7% with placebo; $P<0.001$) in patients undergoing urgent or elective percutaneous revascularization.¹⁵⁵ A similar reduction in the risk of early ischemic events was demonstrated with tirofiban, a synthetic, nonpeptide IIb/IIIa inhibitor, after high-risk coronary angioplasty¹⁵⁶ and with abciximab after intracoronary stenting.¹⁵⁷

The benefit of platelet inhibition in patients with unstable angina has been assessed recently by monitoring troponin T release, which serves as a surrogate marker for thrombus formation. Patients with refractory unstable angina and elevated troponin T levels were shown to constitute a high-risk subgroup who particularly benefited from antiplatelet therapy with abciximab.¹⁵⁸ When added to treatment with intravenous heparin in patients with unstable angina, treatment with intravenous eptifibatide (integrelin), a peptide IIb/IIIa inhibitor, decreased the incidence and duration of ischemic episodes noted on 24-hour ECG monitoring compared with aspirin therapy.¹⁵⁹ In patients with unstable angina or non-Q-wave MI, the addition of tirofiban to aspirin therapy (325 mg/d) reduced the composite end point of death, MI, or refractory ischemia by 32% after 48 hours of therapy (3.8%

versus 5.6% with heparin; $P=0.01$)¹⁶⁰; however, at 30 days, the difference was no longer significant. In a group of patients with more severe unstable angina and a higher proportion of non-Q-wave MI, treatment with aspirin plus tirofiban resulted in an increase in mortality compared with a regimen of aspirin plus intravenous heparin (mortality rate of 4.6% versus 1.1% at 7 days; $P=0.012$).¹⁶¹ However, the addition of tirofiban to a regimen of aspirin plus heparin decreased the composite end point of death, MI, or refractory ischemia at 7 days by 32% (12.9% versus 17.9%; $P=0.004$). This benefit persisted, although to a smaller degree, at 30 days and at 6 months after treatment.

Taken together, these trials demonstrate a significant benefit of glycoprotein IIb/IIIa inhibitors when administered in addition to usual aspirin therapy in patients with unstable coronary syndromes and after percutaneous revascularization. Although initial studies were complicated by increased rates of bleeding,¹⁵⁴ with adjusted heparin dosing, the expected bleeding rate is not different from that with standard heparin and aspirin therapy.^{155,160,161}

Conclusions

Aspirin clearly decreases mortality and reinfarction when given as short-term therapy for AMI, when given to patients with unstable angina, and when given as long-term secondary preventive therapy in a wide range of patients with established cardiovascular disease. Despite the strength of the data in this regard, studies suggest that aspirin remains underused for both the treatment of acute coronary syndromes^{162,163} and for secondary prevention of recurrent events.^{164–166} More than 10% of patients suffering an AMI do not receive aspirin therapy despite the absence of contraindications,¹⁶² and 20% to 50% of postinfarction patients may not be taking aspirin on an ongoing basis.^{164,165} The statistics are even worse in the elderly population: almost 30% of Medicare patients hospitalized for unstable angina are not treated with aspirin in the short term,¹⁶³ and as many as 80% of nursing home patients with a prior history of MI may not be given aspirin.¹⁶⁶ Nonetheless, its use in these settings should be the accepted standard unless absolute contraindications exist. The dose of aspirin should always be the lowest dose that is known to be effective (ie, 160 to 325 mg for acute treatment of cardiovascular events and 75 to 160 mg/d for primary and secondary prevention) because higher doses result in higher rates of complications. The role of aspirin in primary prevention is less clear. In patients felt to be at high risk of a future cardiac event owing to the presence of significant risk factors, prophylactic aspirin should be considered but weighed against the risk of potential complications. In patients at low risk of cardiac events, the risk of hemorrhagic complications may outweigh the benefits of therapy, and the current data do not support the use of prophylactic aspirin therapy in this setting. As newer aspirin regimens are developed that have improved safety profiles, the risk/benefit ratio may change to support the use of aspirin as primary prevention in a broader range of patients. Other antithrombotic agents, especially the glycoprotein IIb/IIIa inhibitors, which are capable of more complete platelet inhibition, are likely to play an increasingly greater role in the treatment of cardiovascular diseases;

however, given its relative safety and extremely low cost, aspirin will continue to be an important agent in the treatment and prevention of cardiovascular diseases for the foreseeable future.

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Exhibit 13

Effectiveness of Clopidogrel and Aspirin Versus Ticlopidine and Aspirin in Preventing Stent Thrombosis After Coronary Stent Implantation

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Background—Ticlopidine has been shown to reduce the incidence of stent thrombosis compared with warfarin, but it may cause serious hematological side effects. Clopidogrel, a new thienopyridine derivative, may be a safe alternative to ticlopidine. The aim of this study was to compare the safety and efficacy of clopidogrel and aspirin with those of ticlopidine and aspirin in patients undergoing coronary stent implantation.

Methods and Results—The population of this study consisted of 2 groups: patients who underwent coronary stenting and were treated with ticlopidine and aspirin (TA group, $n=1406$), and patients who underwent coronary stenting followed by treatment with clopidogrel and aspirin (CA group, $n=283$). At 1-month follow-up, there was no difference in stent thrombosis (1.5% versus 1.4%, $P=1.0$) or major adverse cardiac events (3.1% versus 2.4%, $P=0.85$) between the TA and CA groups, respectively. The probability of any side effect (neutropenia, diarrhea, rash) was significantly higher in the TA group (10.6% versus 5.3%, $P=0.006$; relative risk, 0.53; CI, 0.32 to 0.86).

Conclusions—These data suggest that clopidogrel may be an effective pharmacological regimen after coronary stent implantation. Furthermore, the simpler dosing regimen, the absence of neutropenia, and the lower frequency of other side effects make it a safe alternative to ticlopidine. (*Circulation*. 1999;99:2364-2366.)

Key Words: clopidogrel ■ ticlopidine ■ stents

Coronary stent implantation has become the dominant form of catheter-based coronary interventions on the basis of data demonstrating the efficacy and safety of coronary stenting when appropriate technique and postprocedure antiplatelet therapy are used.^{1,2} The combination of ticlopidine and aspirin has been confirmed to be superior to aspirin alone or aspirin and coumarin in randomized trials.^{3,4} Despite the effectiveness of ticlopidine, a small incidence of side effects remains,⁵ in particular hematological side effects that may occasionally be fatal.⁶

Clopidogrel, a new thienopyridine derivative, was recently approved for use in patients with atherosclerotic vascular disease to reduce the incidence of ischemic events.⁷ This antiplatelet agent may potentially be of use after stent implantation to reduce stent thrombosis without the added risks of hematological toxicity. As of this writing, few data are available as to the effectiveness of this agent in preventing thrombosis after stenting. The purpose of this study was to compare the safety and effectiveness of clopidogrel and aspirin with those of ticlopidine and aspirin in a consecutive series of patients undergoing coronary stent implantation.

Methods

Patient Population, Stent Implantation, and Pharmacological Regimen

Between September 1996 and June 1998, 2057 patients underwent stent implantation for obstructive coronary artery disease. Of these, 368 were excluded from this study because of (1) requirement for oral anticoagulation (57 patients); (2) administration of abciximab (280 patients); (3) procedural failure: less than TIMI 3 flow (8 patients), residual diameter stenosis $>50\%$ (4 patients), emergency bypass surgery (3 patients), or intracerebral hemorrhage (1 patient); and (4) patients who received aspirin alone (14 patients) or ticlopidine alone (1 patient) because of known allergy to the other agent. The final study population consisted of patients who underwent coronary stenting between September 1996 and February 1998 and were treated with ticlopidine and aspirin (TA group: 1406 patients, 1763 lesions) and patients who underwent coronary stenting between March 1998 and June 1998 and were treated with clopidogrel and aspirin (CA group: 283 patients, 376 lesions). Stent implantation was performed by use of techniques previously described,² and quantitative angiographic analysis was performed with a computer-based system (CMS version 3.0, MEDIS).

Ticlopidine was administered as a loading dose of 500 mg followed by 250 mg PO twice a day for 2 weeks.⁸ Clopidogrel was administered as a loading dose of 300 mg followed by 75 mg PO once a day for 4 weeks. Aspirin was administered as 325 mg PO

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TABLE 1. Patient Clinical and Angiographic Characteristics

Patients	TA Group (n=1406)	CA Group (n=283)	P
Age, y	63±12	64±12	0.20
Male, n (%)	951 (68)	193 (68)	0.89
Diabetes mellitus, n (%)	301 (21)	63 (22)	0.75
Unstable angina, n (%)	490 (35)	97 (34)	0.89
Lesions	(n=1763)	(n=376)	
Lesion complexity,* n (%)			0.08
A	190 (11)	24 (6)	
B1	438 (25)	96 (25)	
B2	586 (33)	130 (35)	
C	549 (31)	126 (34)	
Quantitative angiography			
Preprocedure			
Reference diameter, mm	2.83±0.67	2.78±0.59	0.18
Minimum lumen diameter, mm	0.84±0.47	0.90±0.45	0.02
Lesion length, mm	10.93±5.59	11.63±6.18	0.05
Postprocedure			
Minimum lumen diameter, mm	3.13±0.55	3.08±0.48	1.0
Diameter stenosis, %	10±15	10±3	1.0

A value of $P<0.05$ is considered significant.

*AHA/ACC classification.

once a day to both groups. All patients were instructed to follow up with their referring physician in 2 weeks for clinical assessment and blood count analysis. In addition, a dedicated nurse practitioner (R.M.) was performing telephonic follow-up evaluation at 1 month on an ongoing basis.

Statistics

Statistical analysis was performed with StatView software. Continuous normally distributed data were expressed as mean±SD and were compared by unpaired Student's *t* test. Categorical variables were expressed as numbers and percentages and compared by the χ^2 test. Differences were considered statistically significant at a value of $P<0.05$.

Results

Patient Characteristics and Procedural Data

Patient characteristics and angiographic measurements are shown in Table 1. Similar numbers (1.3±0.6 versus 1.3±0.5, $P=1.0$) and types (slotted tube, 87% versus 88%; coil, 12% versus 12%; and Wallstent, 1% versus 0%, $P=0.49$) of stents were implanted in the TA and CA groups, respectively. Bailout stenting was performed with similar frequency in both groups (6% versus 7%, $P=0.57$).

Medication Side Effects and Clinical Outcome

During the period of this study, 16 patients in the TA group (1.1%) and 2 patients in the CA group (0.7%) were lost to follow-up. Of the study population, 46 patients (3.3%) in the TA group and 8 patients (2.8%) in the CA group ($P=0.85$) discontinued the study drug early for reasons other than the occurrence of an outcome event. Reasons for stopping ticlopidine were rash in 30 patients, diarrhea in 6 patients, rash and diarrhea in 5 patients, neutropenia in 4 patients, and noncompliance in 1 patient. Reasons for stopping clopidogrel

TABLE 2. Incidence of Stent Thrombosis, Major Adverse Cardiac Events, and Drug Side Effects at 1-Month Follow-Up

Patients	TA Group (n=1390)	CA Group (n=281)	P
Stent thrombosis	21 (1.5)	4 (1.4)	1.0
Myocardial infarction	25 (1.8)	2 (0.7)	0.29
Non-Q-wave	18 (1.3)	2 (0.7)	
Q-wave	7 (0.5)	0 (0)	
Coronary artery bypass surgery	5 (0.4)	2 (0.7)	0.33
Death	12 (0.9)	3 (1)	0.73
Drug side effects			
Neutropenia	4 (0.3)	0 (0)	1.0
Diarrhea	61 (4.4)	9 (3.2)	0.5
Rash	82 (6)	6 (2)	0.008
Any side effect	147 (10.6)	15 (5.3)	0.006

Values are n (%). A value of $P<0.05$ is considered significant.

were rash in 4 patients, diarrhea in 3 patients, and noncompliance in 1 patient. The incidence of stent thrombosis, cardiac events, and medication side effects at 1-month follow-up is shown in Table 2.

Discussion

Rationale for Use of Clopidogrel After Stent Implantation

Clopidogrel is a thienopyridine derivative that inhibits platelet aggregation by inhibiting the binding of ADP to its platelet receptor, which leads to direct inhibition of the binding of fibrinogen to the glycoprotein IIb/IIIa complex.⁹ Although both ticlopidine and clopidogrel prevent platelet aggregation evoked by shear stress, experimental studies suggest that clopidogrel is more effective than either aspirin or ticlopidine in preventing the high-shear-stress-dependent coronary stent thrombosis.¹⁰ Furthermore, clopidogrel has a favorable safety profile compared with ticlopidine, for which routine hematological monitoring is mandatory to ensure early detection of potentially lethal hematological events. The incidence of neutropenia with ticlopidine is proportional to the duration of treatment (up to 2.4%), and it may resolve with drug cessation in most but not all cases.⁵ Another serious side effect of ticlopidine is thrombotic thrombocytopenic purpura (TTP). A recent review⁶ documented 60 cases of TTP among patients treated with ticlopidine, with an associated mortality rate of 33%. In this review, 12 patients developed TTP after receiving ticlopidine for ≤ 3 weeks after stent implantation. Other common but less morbid adverse effects reported to accompany ticlopidine use are gastrointestinal symptoms.⁵ Clopidogrel was developed because it did not show bone marrow toxicity in tissue culture and animal models. In the large CAPRIE trial,⁷ the incidence of severe neutropenia with long-term use was only 0.05%, which was similar to the rate seen with aspirin (0.04%). In addition, the proportions of patients with severe rash and diarrhea while on clopidogrel in this trial were less than those reported with ticlopidine but twice as high as with aspirin. Therefore, the combination of a favorable safety profile and a proven experimental and

clinical antiplatelet effect make clopidogrel an attractive alternative to ticlopidine after coronary stent implantation.

Clopidogrel: Administration and Clinical Impact

The inhibition of platelet aggregation by clopidogrel is concentration dependent.⁹ In this study, clopidogrel was administered as a loading dose of 300 mg, a dose that provides 80% platelet inhibition in 5 hours,¹¹ followed by 75 mg PO daily for 4 weeks. Aspirin was added to clopidogrel because this drug has no effect on the cyclooxygenase pathway, and therefore, both agents may work synergistically, as is the case with ticlopidine.¹² In this study, stent thrombosis occurred with similar frequency in the ticlopidine and clopidogrel groups (1.5% versus 1.4%, $P=NS$). Similarly, there was no difference between the 2 groups in incidence of major adverse cardiac events at 1-month follow-up (3.1% versus 2.4%, $P=NS$).

With respect to side effects, neutropenia occurred in 4 patients (0.3%) in the ticlopidine group but none of the patients in the clopidogrel group (0%). This rate of neutropenia is similar to those in other stent trials in which ticlopidine was used for 4 weeks.^{3,4} In this study, rash occurred significantly more often in the ticlopidine group despite the short duration of administration. The incidence of diarrhea was also slightly higher, but not statistically significant. Overall, patients in the ticlopidine group were at twice the risk of having any side effect compared with patients receiving clopidogrel.

Study Limitations

First, this is a nonrandomized comparison between the 2 pharmacological regimens. However, these regimens were used in a chronologically consecutive manner, an approach that eliminates the potential for operator bias in selecting one specific regimen over the other. Second, because the incidence of stent thrombosis with antiplatelet therapy is very low, a higher number of patients is necessary to establish equivalence between clopidogrel and ticlopidine. Therefore, a large randomized trial is needed to establish the validity of these data.

Conclusions

These data suggest that clopidogrel may be an effective pharmacological regimen after coronary stent implantation.

Furthermore, the simpler dosing regimen and the absence of hematological toxicity make it a potential safe alternative to ticlopidine.

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Exhibit 14

Articles

A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE)

CAPRIE Steering Committee*

Summary

Background Many clinical trials have evaluated the benefit of long-term use of antiplatelet drugs in reducing the risk of clinical thrombotic events. Aspirin and ticlopidine have been shown to be effective, but both have potentially serious adverse effects. Clopidogrel, a new thienopyridine derivative similar to ticlopidine, is an inhibitor of platelet aggregation induced by adenosine diphosphate.

Methods CAPRIE was a randomised, blinded, international trial designed to assess the relative efficacy of clopidogrel (75 mg once daily) and aspirin (325 mg once daily) in reducing the risk of a composite outcome cluster of ischaemic stroke, myocardial infarction, or vascular death; their relative safety was also assessed. The population studied comprised subgroups of patients with atherosclerotic vascular disease manifested as either recent ischaemic stroke, recent myocardial infarction, or symptomatic peripheral arterial disease. Patients were followed for 1 to 3 years.

Findings 19 185 patients, with more than 6300 in each of the clinical subgroups, were recruited over 3 years, with a mean follow-up of 1.91 years. There were 1960 first events included in the outcome cluster on which an intention-to-treat analysis showed that patients treated with clopidogrel had an annual 5.32% risk of ischaemic stroke, myocardial infarction, or vascular death compared with 5.83% with aspirin. These rates reflect a statistically significant ($p=0.043$) relative-risk reduction of 8.7% in favour of clopidogrel (95% CI 0.3–16.5). Corresponding on-treatment analysis yielded a relative-risk reduction of 9.4%. There were no major differences in terms of safety. Reported adverse experiences in the clopidogrel and aspirin groups judged to be severe included rash (0.26% vs 0.10%), diarrhoea (0.23% vs 0.11%), upper gastrointestinal discomfort (0.97% vs 1.22%), intracranial haemorrhage (0.33% vs 0.47%), and gastrointestinal haemorrhage (0.52% vs 0.72%), respectively. There were ten (0.10%) patients in the clopidogrel group with significant reductions in neutrophils ($<1.2 \times 10^9/L$) and 16 (0.17%) in the aspirin group.

Interpretation Long-term administration of clopidogrel to patients with atherosclerotic vascular disease is more effective than aspirin in reducing the combined risk of ischaemic stroke, myocardial infarction, or vascular death. The overall safety profile of clopidogrel is at least as good as that of medium-dose aspirin.

Lancet 1996; 348: 1329–39

*Study organisation given at end of paper

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Introduction

There have been several randomised trials of antiplatelet drugs in patients with disorders in which platelet activation is involved.¹ Their purpose was to determine the extent of reduction in various subsequent risks; in particular, risks of ischaemic stroke, myocardial infarction, and death from vascular disease (vascular death). Patients at increased risk of such outcomes included those with atherothrombotic disease such as transient ischaemic attacks or mild stroke, moderate or severe stroke, unstable angina, acute and remote myocardial infarction, and atherosclerotic peripheral arterial disease.^{2,3}

Interpretation of these studies has been inconsistent. Many investigators and practitioners apply the results from a particular subgroup of patients, such as those with transient ischaemic attacks or mild stroke, only to patients with that disorder and not to patients with different atherothrombotic manifestations, although it is both clinically and biologically plausible to assume that similar treatment benefits would extend to them. There is evidence from the Antiplatelet Trialists' Collaboration to support a widespread effect.^{4,5} A meta-analysis of 142 trials, including more than 73 000 high-risk patients in various disease categories, shows clearly that antiplatelet drugs reduce the incidence of a composite outcome of ischaemic stroke, myocardial infarction, and vascular death, the relative-odds reduction being 27%, which is consistent over a wide range of clinical manifestations as well as across subgroups of patients at varying risks within specific clinical subgroups.

Both aspirin^{3,4} and ticlopidine^{2,5} have been shown to be of benefit in placebo-controlled studies. Relative-risk reductions for the composite outcomes of stroke, myocardial infarction, or vascular death were 25% with aspirin and 33% with ticlopidine. In three studies in which aspirin was compared with ticlopidine, the odds reduction, while not statistically significant, favoured ticlopidine by 10%.² However, both drugs have potentially serious adverse effects: gastrointestinal discomfort and bleeding with aspirin;⁴ and bone-marrow depression, rash, and diarrhoea with ticlopidine.⁶

Clopidogrel (Plavix) is a new thienopyridine derivative, chemically related to ticlopidine (figure 1). Its activity in animal models of thrombosis is greater than that of ticlopidine.⁷ Clopidogrel prevents arterial as well as venous thrombosis and reduces atherogenesis in several animal species.^{7,8} Clopidogrel blocks activation of platelets by adenosine diphosphate (ADP) by selectively and irreversibly inhibiting the binding of this agonist to its receptor on platelets, thereby affecting ADP-dependent activation of the GpIIb-IIIa complex, the major receptor for fibrinogen present on the platelet surface.^{9,10} In platelet-aggregation studies, clopidogrel, 75 mg once daily, produces inhibition of ADP-induced platelet

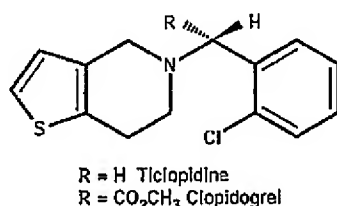


Figure 1: Structure of clopidogrel and ticlopidine
Clopidogrel is the (S) active enantiomer of a racemate.

aggregation equivalent to that of ticlopidine, 250 mg twice daily.

CAPRIE was a randomised clinical trial to assess the potential benefit of clopidogrel, compared with aspirin, in reducing the risk of ischaemic stroke, myocardial infarction, or vascular death in patients with recent ischaemic stroke, recent myocardial infarction, or peripheral arterial disease.

Methods

Protocol

Patient eligibility Clinical evaluation had to establish the diagnosis of ischaemic stroke, myocardial infarction, or symptomatic atherosclerotic peripheral arterial disease. Inclusion and exclusion criteria are shown in tables 1 and 2. Eligible patients who gave informed consent were entered into the study. Use of anticoagulants or antiplatelet drugs was discontinued before randomisation and thrombolytic treatment should not have been received within the previous 48 h. The study protocol was reviewed and approved by the institutional review board or ethics committee of each of the participating centres.

Treatment and follow-up Patients received blister packs containing either 75 mg tablets of clopidogrel plus aspirin placebo or 325 mg tablets of aspirin plus clopidogrel placebo. Patients were asked to take one of each tablet daily with their morning meal. We planned to recruit patients over 3 years with a further year of follow-up and that patients would receive study drugs for a maximum of 3 years and a minimum of 1 year.

Baseline assessment recorded demographic information, the qualifying event or condition, medical history, general physical examination, and concomitant medications. Except in the early stages of the study, follow-up visits took place monthly for the first 4 months and every 4 months thereafter. At these visits, information was collected on adverse events and use of study drug and concomitant medications, and blood was taken for haematological and biochemical assessments by one of three central laboratories. Platelet aggregation testing was forbidden since the results might have revealed treatment allocation. Compliance with study drug was assessed by counting of returned tablets at follow-up visits. Patients were provided with a list of common over-the-counter aspirin-containing products and were instructed to avoid them.

Human safety data on clopidogrel were limited at the start of CAPRIE, so the initial follow-up schedule had weekly assessments of blood counts and 2-weekly assessments of biochemistry during the first 3 months. After 500 patients had been entered, a blinded review of these data by the Steering Committee did not show any cause for concern, so the frequency of these assessments was halved. After data had been collected on the first 1000 patients followed for 3 months, the Steering Committee received a report on these laboratory results prepared by the External Safety and Efficacy Monitoring Committee, classified by treatment A or B, on the basis of which the follow-up schedule was relaxed to that stated above.

Alert values of less than $1.2 \times 10^9/L$ for neutrophils and less than $100 \times 10^9/L$ for platelets were established, whereby investigators were to begin daily complete blood counts. Should the corresponding counts fall below $0.45 \times 10^9/L$ or $80 \times 10^9/L$,

Ischaemic stroke (including retinal and lacunar infarction)	Focal neurological deficit likely to be of atherothrombotic origin
	Onset ≥ 1 week and ≤ 6 months before randomisation Neurological signs persisting ≥ 1 week from stroke onset CT or MRI ruling out haemorrhage or non-relevant disease
Myocardial infarction	Onset ≤ 35 days before randomisation
	Two of: Characteristic ischaemic pain for ≥ 20 min Elevation of CK, CK-MB, LDH, or AST to $2 \times$ upper limit of laboratory normal with no other explanation Development of new ≥ 40 Q waves in at least two adjacent ECG leads or new dominant R wave in V1 (R ≥ 1 mm > S in V1)
Atherosclerotic peripheral arterial disease	Intermittent claudication (WHO: leg pain on walking, disappearing in <10 min on standing) of presumed atherosclerotic origin; and ankle/arm systolic BP ratio ≤ 0.85 in either leg at rest (two assessments on separate days); or history of intermittent claudication with previous leg amputation, reconstructive surgery, or angioplasty with no persisting complications from intervention

CT=computed tomography; MRI=magnetic resonance imaging; CK=creatinine kinase; LDH=lactate dehydrogenase; AST=aspartate aminotransferase; ECG=electrocardiogram; BP=blood pressure; WHO=World Health Organization.

Table 1: Inclusion criteria

respectively, the study drug was to be permanently discontinued. Near the end of the study, all patients for whom a decrease to below the alert value had been reported were reviewed, blind to treatment allocation, by a haematologist to rule out laboratory errors, spoiled samples, and random fluctuations around an inherently low baseline.

Adverse experiences of patients were recorded for the duration of their follow-up, except in those patients who permanently discontinued study drug early; for these patients adverse experiences were counted up to 28 days after discontinuation.

Outcome events Non-fatal events were ischaemic stroke, myocardial infarction, primary intracranial haemorrhage, and leg amputation (table 3). Deaths were classified as due to ischaemic stroke, myocardial infarction, haemorrhage, other vascular causes, or non-vascular causes. The classification of fatal ischaemic stroke or myocardial infarction was based on either death within 28 days after the onset of signs or symptoms of the acute outcome event, in the absence of other clear causes, or on necropsy findings. Other vascular deaths were any deaths that were not clearly non-vascular and did not meet the criteria for fatal stroke, fatal myocardial infarction, or haemorrhage. Deaths considered by the Central Validation Committee to be directly related to the qualifying event were classified as other vascular.

Sample size We planned to recruit 15 000 patients, 5000 in each of the clinical subgroups, over 3 years and to terminate the study

Age <21 years
Severe cerebral deficit likely to lead to patient being bedridden or demented
Carotid endarterectomy after qualifying stroke
Qualifying stroke induced by carotid endarterectomy or angiography
Patient unlikely to be discharged alive after qualifying event
Severe co-morbidity likely to limit patient's life expectancy to less than 3 y
Uncontrolled hypertension
Scheduled for major surgery
Contraindications to study drugs:
Severe renal or hepatic insufficiency
Haemostatic disorder or systemic bleeding
History of haemostatic disorder or systemic bleeding
History of thrombocytopenia or neutropenia
History of drug-induced haematologic or hepatic abnormalities
Known to have abnormal WBC, differential, or platelet count
Anticipated requirement for long-term anticoagulants, non-study antiplatelet drugs or NSAIDs affecting platelet function
History of aspirin sensitivity
Women of childbearing age not using reliable contraception
Currently receiving investigational drug
Previously entered in other clopidogrel studies
Geographic or other factors making study participation impractical
WBC=white blood count; NSAIDs=non-steroidal anti-inflammatory drugs

Table 2: Exclusion criteria

Ischaemic stroke	Acute neurological vascular event with focal signs for ≥ 24 h If in a new location, without evidence of intracranial haemorrhage If worsening of previous event, must have lasted > 1 week, or more than 24 h if accompanied by appropriate CT or MRI findings
Myocardial infarction	As for inclusion criteria (see table 1)
Primary intracranial haemorrhage	Intracerebral haemorrhage (including intracranial and subarachnoid), and subdural haematoma documented by appropriate neuroimaging investigations. (Traumatic intracranial haemorrhage was recorded but not counted as outcome event)
Leg amputation	Only if above the ankle and not done for trauma or cancer. (Subsequent amputations of a given leg were not counted as outcome events)

For abbreviations, see table 1.

Table 3: Non-fatal outcome events

after 1 further year of follow-up. If recruitment over time was uniform, this sample would have resulted in a mean duration of potential follow-up of 2.33 years per patient and 35 000 patient-years at risk. We assumed expected 3-year event rates would be 25% for the primary outcome cluster for patients entering the study with recent stroke or myocardial infarction and 14% for patients entering with peripheral arterial disease. With a two-sided $\alpha=0.05$, the study was expected to have 90% power to detect an overall relative-risk reduction of 11.6%, based on an intention-to-treat analysis. If this were the true effect, the expected width of the corresponding 95% CI would be about 8%.

Patient recruitment was achieved well ahead of schedule and 15 000 patients had been randomised after only 2 years and 3 months. To stop recruitment at that time and close the study after 1 further year of follow-up would have resulted in less than 35 000 potential patient-years at risk. A blinded review of overall outcome event rates showed them to be lower than initial expectations. The Steering Committee decided to continue patient recruitment but to stagger recruitment closing dates and, hence, completion dates, 1 year later: recruitment of patients with peripheral arterial disease would finish 2 months before patients with myocardial infarction who would finish 2 months before patients with stroke. The plan was expected to produce similar numbers of more than 6000 in each of the clinical subgroups and facilitate study closedown. A revised total of 40 000 potential patient-years at risk was expected and the revised estimate of relative-risk reduction that could be detected with 90% power would be 12–13%.

Primary analysis of efficacy was based on the first occurrence of an event in the outcome cluster of ischaemic stroke, myocardial infarction, or vascular death. A secondary outcome cluster included amputation and a further comparison was based on vascular death only. Although the main focus was on events presumed to be due to atherosclerotic disease, primary intracranial haemorrhage and fatal bleeds were possible adverse events, so these were included in an assessment of overall net benefit with the outcome cluster of any stroke, myocardial infarction, or death from any cause. A fourth secondary analysis assessed all-cause mortality.

Assessments of relative efficacy were based on a comparison between the two treatment groups of the cumulative risk over time of each of the five prespecified outcomes. Survival curves based on the proportion of patients remaining event-free were estimated by the Kaplan-Meier method¹¹ and compared by a two-sided Mantel-Haenszel test,^{12,13} stratified by clinical subgroup.

Two analytical strategies were planned: an intention-to-treat analysis in which all patients randomised were considered at risk to their planned end of study, irrespective of their compliance with study protocol, and an on-treatment analysis in which a patient's time at risk was censored 28 days after early permanent discontinuation of study drug. In addition, to take into account any imbalances between the two treatment groups in baseline prognostic variables, analyses were repeated with adjustment procedures based on Cox's proportional hazards model.¹⁴

Primary analysis, however, was to be the unadjusted intention-to-treat comparison based on the outcome cluster of ischaemic stroke, myocardial infarction, or vascular death. Similar analyses were carried out for each of the clinical subgroups.

Safety assessments were based on the proportion of patients experiencing one or more episodes of a specific adverse event. Such proportions in the two treatment groups were compared by χ^2 test.

Patients lost to follow-up In May, 1996 (3 months after the end of the trial) a search agency was contracted by the Coordinating and Methods Centre to help trace patients who were lost to follow-up.

Study organisation

The study involved 384 clinical centres from 16 countries and followed US Investigational New Drug regulations and European Good Clinical Practice guidelines, as well as local requirements. In order to make the most of expertise and resources of both researchers and the industrial backers of the trial, a complex organisation was created.

The *Steering Committee*, comprising university-based and industry-based scientists, had overall responsibility for the design, execution, analysis, and reporting of the study. This committee met every 6 months to address policy issues and to monitor study execution and management. The Steering Committee has responsibility for all publications resulting from the study.

The *Central Validation Committee* was responsible for validating all reported non-fatal outcome events and reported classifications of cause of death, with a secretariat at the *Coordinating and Methods Centre* in Hamilton, Ontario. After an outcome event dossier was received, only the secretariat had any communication with the reporting investigator about the validation of the event. The secretariat maintained a database of validated outcome events, a copy of which was not provided to the industrial backers before the end of the study.

Each reported outcome event was reviewed independently by two members of the Central Validation Committee. Any disagreements between them were resolved by committee review. Committee disagreement with a reported outcome event was made known to the investigator who could either agree with the Committee or provide additional information to support the initial judgment. When agreement could still not be reached, the decision of the Central Validation Committee was final.

The *External Safety and Efficacy Monitoring Committee* had responsibility for monitoring of patient safety and for formal interim analyses of efficacy. This committee had an associated *Independent Statistical Centre* in Lyon, France, that received an updated copy of the study database every 3 months from the Coordinating and Methods Centre. Information on study-drug allocation was merged with study data and routine aggregate safety summaries produced. In addition to safety monitoring, there were to be three interim analyses of efficacy, based on the primary outcome cluster, when 25%, 50%, and 75% of the planned patient-years at risk had accumulated. Stopping guidelines used a Peto-Haybittle type rule based on the p value of the Mantel-Haenszel test. A two-sided type 1 error of 0.001 was used which preserved a type 1 error of 0.048 for the end-of-study analysis. The results of interim analyses were to be disclosed to the Chairman of the Steering Committee only if the stopping rule was met. The quarterly External Safety and Efficacy Monitoring Committee reports also included a futility stopping rule based on the current 95% CI on the relative-risk reduction for the primary outcome cluster; the upper end of the interval had to exceed a 14% relative-risk reduction in favour of clopidogrel compared with aspirin, otherwise the Steering Committee had to be informed. After each quarterly review, a report was sent to the chairman of the Steering Committee stating only that there was no reason not to continue the trial as planned.

The *Coordinating and Methods Centre* at Hamilton facilitated and oversaw the study and provided methodological and administrative support to all committees, investigators, and other study personnel.

Characteristic	All patients		Stroke subgroup		MI subgroup		PAD subgroup	
	Clopidogrel (n=9599)	Aspirin (n=9555)	Clopidogrel (n=3233)	Aspirin (n=3198)	Clopidogrel (n=3143)	Aspirin (n=3159)	Clopidogrel (n=3223)	Aspirin (n=3229)
Mean (SD) age in years	62.5 (11.1)	62.5 (11.1)	64.5 (11.2)	64.7 (11.0)	58.6 (11.4)	58.3 (11.3)	64.2 (9.6)	64.4 (9.7)
% male	72	72	64	63	61	61	73	72
% white	95	95	91	91	96	98	98	98
Percentage of patients with a history of:								
Ischaemic stroke*	9	9	17	19	2	2	6	6
TIA/RIND	10	10	19	19	3	2	8	8
Diabetes mellitus	20	20	26	26	14	15	21	21
Hypertension	52	51	65	65	39	38	61	61
Hypercholesterolaemia	41	41	37	38	41	42	45	45
Angina (stable)	22	22	14	14	25	25	26	27
Angina (unstable)	9	9	3	3	17	17	6	6
Myocardial infarction*	17	16	13	11	17	17	21	21
Congestive heart failure	6	5	4	4	7	7	8	6
Coronary artery disease	5	4	6	5	4	3	4	4
Atrial fibrillation	4	4	4	4	5	5	4	4
Intermittent claudication*	5	4	8	8	5	5
Current cigarette smoker	29	30	22	22	28	29	36	38
Ex cigarette smoker	48	49	43	44	51	50	53	52

*Not including the qualifying event; MI=myocardial infarction; PAD=peripheral arterial disease; TIA=transient ischaemic attack; RIND=reversible ischaemic neurological deficit.

Table 4: Baseline characteristics

Regional Data Collection Centres were at Hamilton, responsible for all the Canadian centres, and in affiliates of industrial backers—one in the USA and two in Europe.

Assignment

The Independent Statistical Centre provided computer-generated balanced blocks of four treatments with random allocation to clopidogrel or aspirin, stratified by clinical centre and the three disease subgroups. Access to this code was restricted to the Independent Statistical Centre, the Chairman of the External Safety and Efficacy Monitoring Committee, and to two independent companies responsible for preparing the study drugs. A copy of the randomisation scheme was deposited with a public notary.

Blinding

Patients were allocated study drugs sequentially from supplies at the clinical centre packaged in a predetermined order in a carton that contained supplies for four patients. These supplies were in the form of blister packs containing either 75 mg tablets of clopidogrel plus aspirin placebo tablets or 325 mg aspirin tablets plus clopidogrel placebo tablets, such blister packs being indistinguishable from one another. The initial supply of study drug had a sealed treatment code label attached which once opened could not be revealed in its original form; this was retained at the clinical centre for emergency code-breaking purposes. There were 21 (0.11%) code breaks during the course of the study, of which 11 were patients in the clopidogrel group and ten in the aspirin group. At the close of the study, a

representative sample of 3358 code-break labels were retrieved and the Independent Statistical Centre verified that there were no code-break labels opened other than those previously reported to them.

Analysis

At the end of the study, the Coordinating and Methods Centre provided a copy of the final study database to the Independent Statistical Centre which, in turn, provided a copy of the randomisation code to the Coordinating and Methods Centre. The Independent Statistical Centre then carried out the primary analysis and four secondary analyses to verify the corresponding analyses conducted by the Coordinating and Methods Centre. A copy of the randomisation scheme was not provided to the industrial backers until after the Steering Committee had met to be apprised of the findings from the study.

Event type	Clopidogrel	Aspirin	Total
Non-fatal events			
Non-fatal ischaemic stroke	472	504	976
Non-fatal MI	255	301	556
Non-fatal primary ICH	14	24	38
Amputation	52	47	99
Fatal events			
Fatal ischaemic stroke	37	42	79
Fatal MI	53	75	128
Haemorrhagic death	23	27	50
Other vascular death	260	261	521
Non-vascular death	187	165	352
Total	1353	1447	2800

MI=myocardial infarction; ICH=intracranial haemorrhage.

Table 5: Validated events

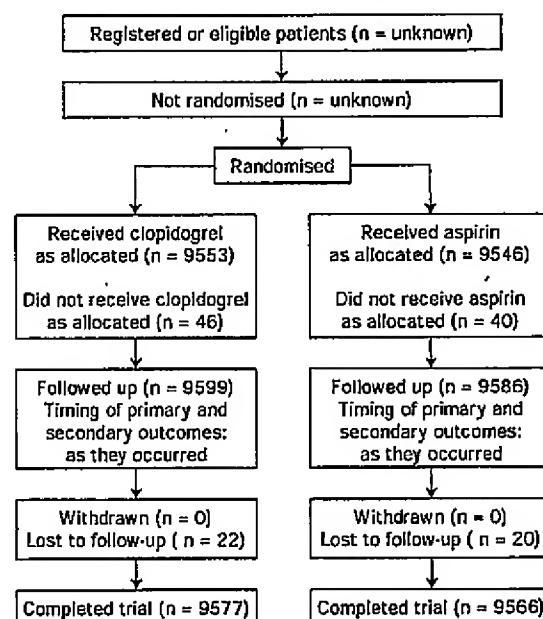


Figure 2: Participant progress through trial

Outcome event cluster and treatment group	First outcome events			Event rate per year	Relative-risk reduction (95% CI)	p
	Non-fatal	Fatal	Total			
Ischaemic stroke, MI, or vascular death (primary cluster)						
Clopidogrel (nys=17636*)	631	308	939	5.32%	8.7% (0.3 to 16.5)	0.043
Aspirin (nys=17619)	700	321	1021	5.83%		
Ischaemic stroke, MI, amputation, or vascular death						
Clopidogrel (nys=17594)	677	302	979	5.56%	7.6% (-0.8 to 15.3)	0.076
Aspirin (nys=17482)	737	314	1051	6.01%		
Vascular death						
Clopidogrel (nys=17482)	..	350	350	1.90%	7.6% (-8.9 to 20.1)	0.28
Aspirin (nys=18354)	..	378	378	2.06%		
Any† stroke, MI, or death from any cause						
Clopidogrel (nys=17622)	643	490	1133	6.43%	7.0% (-0.9 to 14.2)	0.081
Aspirin (nys=17501)	720	487	1207	6.90%		
Death from any cause						
Clopidogrel (nys=18377)	..	560	560	3.05%	2.2% (-9.9 to 12.9)	0.71
Aspirin (nys=18354)	..	571	571	3.11%		

*Patient-years at risk for outcome cluster; †includes primary intracranial hemorrhage; MI=myocardial infarction.

Table 6: Intention-to-treat analysis—primary and secondary outcome clusters

Results

Participants and follow-up

19 185 patients from 384 clinical centres were randomised between March, 1992, and February, 1995. Patient follow-up was completed by February, 1996, resulting in 36 731 patient-years at risk. Mean duration of follow-up was 1.91 years.

During the study, 42 patients (0.22%) were lost to follow-up, 22 in the clopidogrel group and 20 in the aspirin group (figure 2); the resulting loss in total patient-years at risk was 49 (0.13%). These 42 patients were included in the analyses with their follow-up censored at the time of last contact.

4059 patients (21.2%) had study drug permanently discontinued early, for reasons other than the occurrence of an outcome event; 21.3% in the clopidogrel and 21.1% in the aspirin group. Reasons for stopping study drug early were similar in the two groups: adverse events (11.4%); withdrawn consent (4.7%); contraindicated medications (2.4%); non-compliance (1.8%); and other (0.8%). Mean follow-up while on study drug was 1.63 years for each treatment group.

With exclusion of follow-up after any early permanent discontinuation of study drug, mean compliance with clopidogrel and aspirin was similar at 91%. 46 patients in the clopidogrel group and 40 in the aspirin group never took any study drug.

Analysis

Baseline characteristics of randomised patients are shown in table 4. The treatment groups were well matched with respect to age, sex, race, and cardiovascular risk factors. After randomisation, 16 patients, ten in the clopidogrel group and six in the aspirin group, were found not to have the qualifying disease; most were entered as having ischaemic stroke but were subsequently found to be misdiagnosed, (eg, as multiple sclerosis or primary intracranial haemorrhage). The study drug was terminated within 4 months of randomisation for 13 of these patients but the other three patients were continued on study drug; all 16 continued to be followed as per protocol and included in the analyses.

There were 2800 validated outcome events, of which 1669 were non-fatal and 1131 were fatal (table 5). There were 1171 patients in the clopidogrel group and 1236 patients in the aspirin group who had an outcome event of

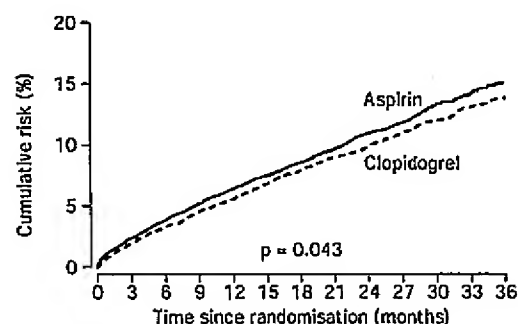
whom 158 and 182, respectively, had more than one event.

The primary analysis of efficacy was by intention-to-treat and based on the incidence of the first occurrence of ischaemic stroke, myocardial infarction, or vascular death among all patients randomised. There were 939 events in the clopidogrel group during 17 636 patient-years at risk, an average rate per year of 5.32%. There were 1021 events in the aspirin group during 17 519 patient-years at risk, an average rate per year of 5.83%. Relative-risk reduction, estimated from a Cox proportional-hazard model, was 8.7% (95% CI 0.3–16.5) in favour of clopidogrel ($p=0.043$, table 6). The cumulative proportions of patients who experienced an event in this primary outcome cluster over 3 years are shown in figure 3.

Results of the analyses of the four predefined secondary outcome clusters are also shown in table 6. The estimated relative-risk reductions with clopidogrel were consistently 7% to 8% when the outcomes were predominantly vascular events but the relative-risk reduction was smaller for all-cause mortality, of which 36% was non-vascular.

Estimated treatment effects for both the primary and secondary outcome clusters remained virtually unchanged when adjusted for relevant prognostic baseline variables.

Main baseline characteristics for each of the subgroups are shown in table 4. Patients in the ischaemic stroke and peripheral arterial disease groups were similar in age and



Patients A: 9586 9190 8087 6139 3979 2143 542
at risk C: 9599 9247 8131 6160 4053 2170 539

Figure 3: Cumulative risk of ischaemic stroke, myocardial infarction, or vascular death
A=aspirin; C=clopidogrel.

Subgroup and treatment group	Individual first-outcome events				Other vascular death	Total	Event rate per year	Relative-risk reduction (95% CI)	p
	Stroke		MI						
	Non-fatal	Fatal	Non-fatal	Fatal					
Stroke									
Clopidogrel (n yrs=6054*)	298	17	33	11	74	433	7.15%	7.3% (-5.7 to 18.7)	0.26
Aspirin (n yrs=5979)	322	16	37	14	72	461	7.71%		
MI									
Clopidogrel (n yrs=5767)	37	5	143	20	66	291	5.03%	-3.7% (-22.1 to 12.0)	0.66
Aspirin (n yrs=5843)	34	8	152	22	67	283	4.84%		
PAD									
Clopidogrel (n yrs=5795)	70	11	50	18	66	215	3.71%	23.8% (8.9 to 36.2)	0.0028
Aspirin (n yrs=5797)	74	8	81	27	87	277	4.86%		
All patients									
Clopidogrel (n yrs=17636)	405	33	225	49	226	939	5.32%	8.7% (0.3 to 16.5)	0.043
Aspirin (n yrs=17519)	430	32	270	63	226	1021	5.83%		

*Patient years at risk. MI=myocardial infarction; PAD=peripheral arterial disease.

Table 7: Treatment effect by subgroup—Ischaemic stroke, MI, or vascular death

years older on average than those in the myocardial infarction group, and there were differences in the proportion of men across the three clinical subgroups. Previous history of vascular events and vascular risk factors show that there was an overlap in the three clinical subgroups. For example, 12% of the stroke subgroup and 8% peripheral arterial disease reported a history of myocardial infarction. 2% of the younger myocardial infarction subgroup reported previous stroke and 6% peripheral arterial disease. 6% of the peripheral arterial disease group had experienced a previous stroke and 21% a previous myocardial infarction. About 18% of the stroke subgroup had experienced at least one additional stroke before their qualifying event; similarly the qualifying myocardial infarction was not their first for 17% of the myocardial infarction subgroup. 50% of the study cohort had a history of hypertension, 25% had a history of angina, and 20% had diabetes mellitus.

For the ischaemic stroke group, mean time from stroke onset to randomisation was 53 days; 59% of qualifying events were atherothrombotic and 40% lacunar. For the myocardial infarction group, mean time from onset of symptoms to randomisation was 17.6 days, 34% of the qualifying events were anterior and 57% were inferior. For the peripheral arterial disease group, mean duration of symptomatic disease before randomisation was 4.2 years and 63% were eligible on the basis of arterial intervention. For those qualifying on the basis of current claudication, the mean ankle/arm blood pressure ratio at entry was 0.57. These baseline characteristics were similar between the two treatment groups.

Analyses based on the primary outcome cluster of ischaemic stroke, myocardial infarction, or vascular death are summarised for each of the clinical subgroups in table 7, which also shows the type of first outcome event. Within this primary cluster of ischaemic events, recurrent stroke and stroke deaths were most common within the stroke subgroup and fatal or non-fatal myocardial infarctions most common in the myocardial infarction subgroup. Patients with peripheral arterial disease had approximately equal risks of stroke and myocardial infarction.

For patients with stroke, the average event rate per year in the clopidogrel group was 7.15% compared with 7.71% in the aspirin group, a relative-risk reduction of 7.3% (-5.7 to 18.7) in favour of clopidogrel ($p=0.26$). For patients with myocardial infarction, the average event rate per year was 5.03% in the clopidogrel group compared with 4.84% in the aspirin group; a relative-risk increase of 3.7% (22.1 to -12.0) associated with clopidogrel ($p=0.66$). For patients with peripheral arterial disease, the average event rate per year in the clopidogrel group was 3.71% compared with 4.86% in the aspirin group; a relative-risk reduction of 23.8% (8.9 to 36.2) in favour of clopidogrel ($p=0.0028$) (figure 4).

A test of heterogeneity of these three treatment effects, was statistically significant ($p=0.042$), suggesting that the true benefit may not be identical across the three clinical subgroups.

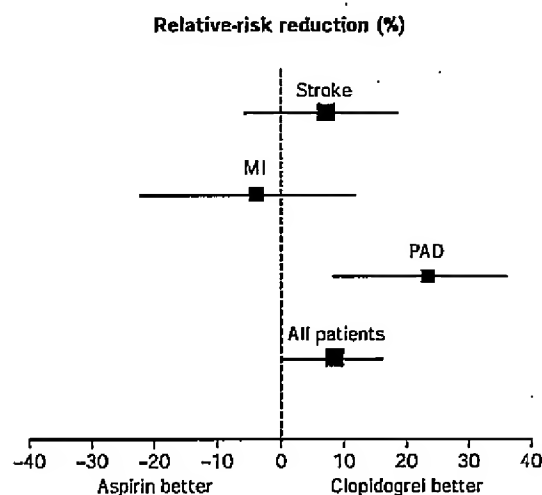


Figure 4: Relative-risk reduction and 95% CI by disease subgroup
MI=myocardial infarction; PAD=peripheral arterial disease.

Patient and treatment subgroup	Ischemic stroke, MI, or vascular death		Relative-risk reduction (95% CI)
	Events	Rate/yr	
PAD/stroke with previous MI (n=2144*)			
Clopidogrel (n=19631)	164	8.35%	22.7% (4.9 to 37.2)
Aspirin (n=1825)	196	10.74%	
Any previous MI (n=8446)			
Clopidogrel (n=7751)	455	5.87%	7.4% (-5.2 to 19.6)
Aspirin (n=7668)	479	6.25%	

PAD=peripheral arterial disease; MI=myocardial infarction; *Number of patients; †Number of patient years at risk

Table 8: Treatment effects of patients with a history of MI

Corresponding estimates of treatment effects based on the on-treatment analysis (while on study drug or within 28 days of early permanent discontinuation) were also made. Most of the on-treatment analyses yielded slightly increased treatment effects. In particular, the relative-risk reduction of 8.7% on the primary outcome cluster increased to 9.4% in on-treatment analysis.

The lack of observed benefit of clopidogrel over aspirin in the myocardial infarction subgroup and the evidence of possible heterogeneity of treatment effect among the clinical subgroups prompted a single additional analysis.

There were 2144 patients in the ischaemic stroke and peripheral arterial disease groups with a previous history of myocardial infarction. For the primary outcome cluster of ischaemic stroke, myocardial infarction, or vascular death, the average event rate per year in the clopidogrel group was 8.35% compared with 10.74% in the aspirin group, a relative-risk reduction of 22.7% (4.9-37.3) in favour of clopidogrel (table 8). When this group with a history of myocardial infarction is combined with the myocardial infarction (qualifying event) subgroup, the overall relative-risk reduction in favour of clopidogrel becomes 7.4% (-5.2 to 18.6).

There was no evidence of any unusual findings of adverse effects in either treatment group. Table 9 shows the proportion of patients ever reporting adverse experiences, those judged by the investigator to be clinically severe, and those which were sufficient to result in early permanent discontinuation of study drug. Liver function was monitored routinely, given the experimental stage of clopidogrel; no treatment-related effect was seen. The frequency of severe rash was higher with clopidogrel than with aspirin ($p=0.017$) as was the frequency of severe diarrhoea ($p=0.080$). More frequent with aspirin were severe upper gastrointestinal discomfort ($p=0.096$), intracranial haemorrhage ($p=0.23$), and gastrointestinal haemorrhage ($p=0.05$). There were also more patients with validated non-fatal primary intracranial haemorrhage or haemorrhagic death in the aspirin group, (51 [0.53%] vs 37 [0.39%]). There were no clinically significant

changes over time in the various laboratory measures, in particular for plasma cholesterol concentrations.

The independent blinded haematological review found the number of cases below the platelet-alert value was 25 (0.26%) in the clopidogrel group and 25 (0.26%) in the aspirin group; the numbers for low neutrophil counts were ten (0.10%) and 16 (0.17%). Among these latter cases, the neutrophil count fell below $0.45 \times 10^9/L$ for five (0.05%) and four (0.04%) patients in the clopidogrel and aspirin groups, respectively.

Discussion

CAPRIE is the first study of an antiplatelet drug to include patients from the clinical subgroups of ischaemic cerebrovascular, cardiac, and peripheral arterial disease under a common protocol. We reasoned from available evidence that in a study on prevention, separations within and amongst clinical subgroups are not necessary because the underlying condition is atherothrombosis which can become clinically manifest in different ways. This approach can be justified by the common aetiology because many patients have experienced one manifestation when they present to medical attention with another; because of the consistency of the effect of antiplatelet drugs across clinical subgroups; and because in necropsy studies, many patients who have atherosclerosis in one part of the body are found to have it in others.

CAPRIE was powered to detect a realistic treatment effect in the whole study cohort but not in each of the three clinical subgroups. The intention-to-treat analysis of the primary outcome cluster showed an overall relative-risk reduction of 8.7% ($p=0.043$), with 95% CI of 0.3-16.5. When the corresponding subgroup analyses were carried out separately for the ischaemic stroke, myocardial infarction, and peripheral arterial disease subgroups, the estimated relative-risk reductions were 7.3%, -3.7%, and 23.8%, respectively. A test for heterogeneity was significant ($p=0.042$) suggesting that the observed differences in these relative treatment effects were greater than might be due to chance. From these observed treatment effects, the possibility cannot be ruled out entirely that clopidogrel and aspirin are only equivalent in benefit in patients presenting with myocardial infarction or that the benefit of clopidogrel over aspirin is truly much greater in patients with peripheral arterial disease.

To help interpret the apparently discrepant finding in the myocardial infarction subgroup, an additional analysis, not specified in the protocol, was considered relevant because aspirin has similar benefits in preventing major ischaemic events in patients with acute myocardial infarction and in those with a remote history of myocardial infarction.³ There were 2144 patients in the stroke and peripheral arterial disease groups who had a distant past

Adverse experience	Patients ever reporting		Severe		Study drug permanently discontinued	
	Clopidogrel	Aspirin	Clopidogrel	Aspirin	Clopidogrel	Aspirin
Rash	578 (0.02%)	442 (4.61%)*	25 (0.28%)	10 (0.10%)*	85 (0.90%)	39 (0.41%)*
Diarrhoea	428 (4.46%)	322 (3.36%)*	22 (0.23%)	11 (0.11%)	40 (0.42%)	26 (0.27%)
Indigestion/nausea/vomiting	1441 (15.01%)	1686 (17.59%)*	93 (0.97%)	118 (1.23%)	182 (1.90%)	231 (2.41%)*
Any bleeding disorder	890 (9.27%)	890 (9.28%)	132 (1.38%)	149 (1.55%)	115 (1.20%)	131 (1.37%)
Intracranial haemorrhage	34 (0.35%)	47 (0.49%)	30 (0.31%)	41 (0.43%)	20 (0.21%)	32 (0.33%)
Gastrointestinal haemorrhage	191 (1.99%)	255 (2.66%)*	47 (0.49%)	68 (0.71%)*	50 (0.52%)	89 (0.93%)*
Abnormal liver function	285 (2.97%)	302 (3.15%)*	11 (0.11%)	9 (0.09%)	22 (0.23%)	28 (0.29%)

*Statistically significant, $p<0.05$.

Table 9: Adverse experiences (number and percentage of patients)

history of myocardial infarction. When this cohort was combined with the 6302 patients who presented with myocardial infarction as the qualifying event, the overall relative-risk reduction was 7.4% (–5.2 to 18.6) in favour of clopidogrel, consistent with the observed benefit in the rest of the CAPRIE cohort.

The Antiplatelet Trialists' Collaboration provides strong evidence that long-term use of antiplatelet drugs results in a relative-risk reduction in ischaemic stroke, myocardial infarction, or vascular death, which is consistent across these three clinical subgroups.³ Given this finding and the additional analysis, we judge that the weak evidence of heterogeneity does not invalidate the underlying concept in CAPRIE.

The observed 3-year event rates in the aspirin group for the stroke and peripheral arterial disease subgroups were close to those postulated at the start but were lower in the myocardial infarction subgroup (13 vs 25%). The reasons for this are not clear. It is possible that patient selection was influenced by competing trials in acute myocardial infarction or by investigators keeping those patients with larger infarcts out of this trial in order to give open-label aspirin or anticoagulants. The lower rate may also be due to recent improvements in acute management of patients.

Bias resulting from study execution is unlikely since the blinding was well maintained, the numbers of patients lost to follow-up and treatment code breaks at the clinical centres were small, and the rate of early permanent discontinuation of study drug was lower than reported in similar studies. The central validation of all reported outcome events provided a consistent assessment and should enhance the credibility of the efficacy findings.

Bleeding is a complication of antiplatelet treatment.⁴ Reported severe bleeding was more common with aspirin, with the difference in severe gastrointestinal bleeding being statistically significant. Non-fatal primary intracranial haemorrhage and haemorrhagic deaths were predefined outcome events that could possibly be caused by study drug. These were less frequent in the clopidogrel group (0.39%) than in the aspirin group (0.53%).

Clopidogrel is a thienopyridine derivative, as is ticlopidine. Ticlopidine is known to cause neutropenia (neutrophils less than $1.2 \times 10^9/L$) for which the reported rate of occurrence is about 2.4% and severe neutropenia (less than $0.45 \times 10^9/L$) for which the reported frequency is 0.8%.⁶ In CAPRIE, there was no excess neutropenia in the clopidogrel group. The observed frequency of neutropenia was 0.10% with clopidogrel and 0.17% with aspirin; for severe neutropenia, the corresponding rates were 0.05% and 0.04%. The proportions of patients with severe rash and diarrhoea while on clopidogrel were less than those reported with ticlopidine but twice as high as with aspirin. Although these latter two differences between clopidogrel and aspirin are statistically significant, the absolute difference of about 0.1% is unlikely to be clinically important, and is balanced by the extent of upper gastrointestinal discomfort with aspirin.

Clopidogrel provides an additional 8.7% relative-risk reduction over and above the 25% reduction accepted to be provided by aspirin. Thus, in a patient population similar to that in CAPRIE, aspirin would be expected to prevent about 19 major clinical events versus 24 with clopidogrel, for each 1000 patients treated for 1 year. The efficacy results from CAPRIE are consistent with the previous findings with ticlopidine and indicate that thienopyridines have a greater benefit than aspirin in

patients with atherothrombotic disease, confirming the importance of the ADP pathway, compared with the thromboxane pathway, in this disease. This benefit was achieved with no evidence of excess neutropenia, a risk of clinically relevant bleeding less than that with 325 mg aspirin per day, and no other toxicity of concern.

Clopidogrel is at least as safe as medium-dose aspirin and is safer than ticlopidine. Given this favourable efficacy/safety ratio, clopidogrel is an effective new antiplatelet agent for use in atherothrombotic disease.

CAPRIE Study Organisation

Steering Committee: M Gent (Chairman), D Beaumont, J Blanchard, M-G Bousser, J Coffman, J D Easton, J R Hampton, L A Harker, L Janzon, J J E Kusmierz, E Panak, R S Roberts, J S Shannon, J Sicurella, G Tognoni, E J Topol, M Verstraete, C Warlow.

Central Validation Committee: M Verstraete (Chairman), J D Easton (V-Chairman), M-G Bousser, J A Cairns, J H Chesebro, J R Hampton, G von der Lippe, R W Ross Russell, P A Wolf.

External Safety and Efficacy Monitoring Committee: J-P Boissel (Chairman), L Friedman, V Fuster, M G Harrison, S Pocock, B B Weksler.

Hamilton Civic Hospitals Research Centre/McMaster University (Canada):

Coordinating and Methods Centre: M Gent, G Foster, G Lewis, T Lychak, H L Nelson, R S Roberts, J Sicurella.

Secretariat for the Central Validation Committee: J Sicurella, C Stewart, B Szechman.

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Sanofi Winthrop:

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Operational Group and RDCCs: M O Besnier, A Boddy, D Brooker, G Derzko, A Jones, C Metzinger, J Novack, S Pratt, D Roome, J P Schulhof, E Vallee, D Vaiter.

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Collaborating Clinical Centres listed by country in order of number of patients enrolled.

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West of Scotland Coronary Prevention Study: identification of high-risk groups and comparison with other cardiovascular intervention trials

West of Scotland Coronary Prevention Group*

Summary

Background We assessed the potential benefit of treatment for low-risk and high-risk groups in the West of Scotland Coronary Prevention Study (WOSCOPS) population, and compared the benefits of primary and secondary prevention of coronary heart disease (CHD) by lipid lowering with the benefits of blood pressure reduction in the primary prevention of stroke.

Methods We did a subgroup analysis of placebo-treated men in the WOSCOPS population by age, vascular disease at trial entry, and other established risk factors. We also compared WOSCOPS findings with those of the Scandinavian Simvastatin Survival Study (4S) and the Medical Research Council (MRC) trial of treatment for mild to moderate hypertension in middle-aged men. The WOSCOPS population comprised 6595 men aged 45-64 years with no history of myocardial infarction (MI) and plasma total cholesterol concentrations of 6.5-8.0 mmol/L at initial screening. Participants were randomly allocated pravastatin (40 mg daily) or placebo, and followed up for an average of 4.9 years.

Findings Coronary event rates at 5 years in the WOSCOPS placebo group were higher than 10% (the recommended treatment threshold) in men with pre-existing vascular disease and in those 55 years or older without symptoms or signs of CHD but with at least one other risk factor. Event rates were low in men with hypercholesterolaemia but no other risk factor: 3.5% (95% CI 1.3-5.7) for men aged 45-54 years and 5.3% (2.7-8.0) for men aged 55-64 years. Three times more men had to be treated for 5 years to prevent one endpoint in WOSCOPS than in 4S. By contrast, two to four times fewer men with hyperlipidaemia were treated to save one coronary event in WOSCOPS than hypertensives to save one stroke in the MRC trial. These differences persisted after adjustment for the low-risk status of many of the patients with hypertension who took part in the MRC trial.

Interpretation There were a substantial number of men whose risk of a coronary event was more than 10% at 5 years in the WOSCOPS cohort. The absolute benefit of pravastatin treatment of hyperlipidaemia is less in the primary prevention of CHD than in secondary prevention, but is similar to that for primary prevention of stroke by treatment of mild to moderate hypertension in middle-aged men.

Lancet 1996; **348**: 1339-42

Introduction

After publication of the West of Scotland Coronary Prevention Study (WOSCOPS)¹ of pravastatin in men with hypercholesterolaemia we decided to re-examine the role of lipid-lowering drugs in the prevention of coronary heart disease (CHD). WOSCOPS showed that in men aged 45-64 years who had raised serum cholesterol (6.5-8.0 mmol/L), but no previous myocardial infarction (MI), pravastatin treatment reduced the relative risk of non-fatal MI or death definitely attributable to CHD by 31%, that of death definitely or probably related to CHD by 33%, that of death from all cardiovascular causes by 32%, and that of death from any cause by 22%. The absolute risks of these endpoints at 5 years were reduced by 2.4%, 0.6%, 0.7%, and 0.9%, respectively. The proportionate benefit from pravastatin was similar in all subgroups of patients.¹

The findings of WOSCOPS could, in theory, be applied to a substantial proportion of many populations² and would lead to widespread drug treatment. However, this approach may not be desirable or economically feasible because of the constraints on modern health-care systems. Our examination of the issue of the benefit of treatment starts from the position that there is a wide range of absolute risk for CHD morbidity and mortality in our cohort; with a uniform proportionate risk reduction, there should be a corresponding variation in the absolute benefit of treatment.

As a precursor to a detailed examination of cost benefit, we did a subgroup analysis of the WOSCOPS population to identify the characteristics of the men at the highest absolute risk of CHD. We also compared the effect of cholesterol lowering on primary prevention of CHD (as

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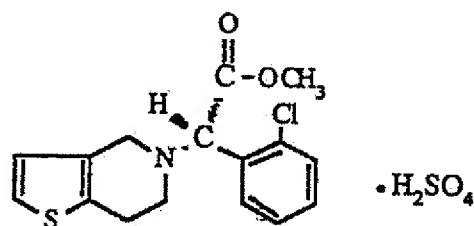
Exhibit 15

PLAVIX®
clopidogrel bisulfate tablets

DESCRIPTION

PLAVIX (clopidogrel bisulfate) is an inhibitor of ADP-induced platelet aggregation acting by direct inhibition of adenosine diphosphate (ADP) binding to its receptor and of the subsequent ADP-mediated activation of the glycoprotein GPIIb/IIIa complex. Chemically it is methyl (+)-(S)- α -(2-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetate sulfate (1:1). The empirical formula of clopidogrel bisulfate is $C_{16}H_{16}ClNO_2S \cdot H_2SO_4$ and its molecular weight is 419.9.

The structural formula is as follows:



Clopidogrel bisulfate is a white to off-white powder. It is practically insoluble in water at neutral pH but freely soluble at pH 1. It also dissolves freely in methanol, dissolves sparingly in methylene chloride, and is practically insoluble in ethyl ether. It has a specific optical rotation of about +56°.

PLAVIX for oral administration is provided as pink, round, biconvex, engraved film-coated tablets containing 97.875 mg of clopidogrel bisulfate which is the molar equivalent of 75 mg of clopidogrel base.

Each tablet contains anhydrous lactose, hydrogenated castor oil, microcrystalline cellulose, polyethylene glycol 6000 and pregelatinized starch as inactive ingredients. The pink film coating contains ferric oxide (red), hydroxypropyl methylcellulose 2910, polyethylene glycol 6000 and titanium dioxide. The tablets are polished with Carnauba wax.

CLINICAL PHARMACOLOGY

Mechanism of Action

Clopidogrel is an inhibitor of platelet aggregation. A variety of drugs that inhibit platelet function have been shown to decrease morbid events in people with established atherosclerotic cardiovascular disease as evidenced by stroke or transient ischemic attacks, myocardial infarction, or need for bypass or angioplasty. This indicates that platelets participate in the initiation and/or evolution of these events and that inhibiting them can reduce the event rate.

Pharmacodynamic Properties

Clopidogrel selectively inhibits the binding of adenosine diphosphate (ADP) to its platelet receptor and the subsequent ADP-mediated activation of the glycoprotein GPIIb/IIIa complex, thereby inhibiting platelet aggregation. Biotransformation of clopidogrel is necessary to produce inhibition of platelet aggregation, but an active metabolite responsible for the activity of the drug has not been isolated. Clopidogrel also inhibits platelet aggregation induced by agonists other than ADP by blocking the amplification of platelet activation by released ADP. Clopidogrel does not inhibit phosphodiesterase activity.

Clopidogrel acts by irreversibly modifying the platelet ADP receptor. Consequently, platelets exposed to clopidogrel are affected for the remainder of their lifespan.

Dose dependent inhibition of platelet aggregation can be seen 2 hours after single oral doses of PLAVIX. Repeated doses of 75 mg PLAVIX per day inhibit ADP-induced platelet aggregation on the first day, and inhibition reaches steady state between Day 3 and Day 7. At steady state, the average inhibition level observed with a dose of 75 mg PLAVIX per day was between 40% and 60%. Platelet aggregation and bleeding time gradually return to baseline values after treatment is discontinued, generally in about 5 days.

Pharmacokinetics and Metabolism

After repeated 75-mg oral doses of clopidogrel (base), plasma concentrations of the parent compound, which has no platelet inhibiting effect, are very low and are generally below the quantification limit (0.00025 mg/L) beyond 2 hours after dosing. Clopidogrel is extensively metabolized by the liver. The main circulating metabolite is the carboxylic acid derivative, and it too has no effect on platelet aggregation. It represents about 85% of the circulating drug-related compounds in plasma.

Following an oral dose of ^{14}C -labeled clopidogrel in humans, approximately 50% was excreted in the urine and approximately 46% in the feces in the 5 days after dosing. The elimination half-life of the main circulating metabolite was 8 hours after single and repeated administration. Covalent binding to platelets accounted for 2% of radiolabel with a half-life of 11 days.

Effect of Food: Administration of PLAVIX with meals did not significantly modify the bioavailability of clopidogrel as assessed by the pharmacokinetics of the main circulating metabolite.

Absorption and Distribution: Clopidogrel is rapidly absorbed after oral administration of repeated doses of 75 mg clopidogrel (base), with peak plasma levels (≈ 3 mg/L) of the main circulating metabolite occurring approximately 1 hour after dosing. The pharmacokinetics of the main circulating metabolite are linear (plasma concentrations increased in proportion to dose) in the dose range of 50 to 150 mg of clopidogrel. Absorption is at least 50% based on urinary excretion of clopidogrel-related metabolites.

Clopidogrel and the main circulating metabolite bind reversibly *in vitro* to human plasma proteins (98% and 94%, respectively). The binding is nonsaturable *in vitro* up to a concentration of 100 $\mu\text{g/ml}$.

Metabolism and Elimination: *In vitro* and *in vivo*, clopidogrel undergoes rapid hydrolysis into its carboxylic acid derivative. In plasma and urine, the glucuronide of the carboxylic acid derivative is also observed.

Special Populations

Geriatric Patients: Plasma concentrations of the main circulating metabolite are significantly higher in elderly (≥ 75 years) compared to young healthy volunteers but these higher plasma levels were not associated with differences in platelet aggregation and bleeding time. No dosage adjustment is needed for the elderly.

Renally Impaired Patients: After repeated doses of 75 mg PLAVIX per day, plasma levels of the main circulating metabolite were lower in patients with severe renal impairment (creatinine clearance from 5 to 15 mL/min) compared to subjects with moderate renal impairment (creatinine clearance 30 to 60 mL/min) or healthy subjects. Although inhibition of ADP-induced platelet aggregation was lower (25%) than that observed in healthy volunteers, the prolongation of bleeding time was similar in healthy volunteers receiving 75 mg of PLAVIX per day. No dosage adjustment is needed in renally impaired patients.

Gender: No significant difference was observed in the plasma levels of the main circulating metabolite between males and females. In a small study comparing men and women, less inhibition of ADP-induced platelet aggregation was observed in women, but there was no difference in prolongation of bleeding time. In the large, controlled clinical study (Clopidogrel vs. Aspirin in Patients at Risk of Ischemic Events; CAPRIE), the incidence of clinical outcome events, other adverse clinical events, and abnormal clinical laboratory parameters was similar in men and women.

Race: Pharmacokinetic differences due to race have not been studied.

CLINICAL STUDIES

The clinical evidence for the efficacy of PLAVIX is derived from the CAPRIE (Clopidogrel vs. Aspirin in Patients at Risk of Ischemic Events) trial. This was a 19,185-patient, 304-center, international, randomized, double-blind, parallel-group study comparing PLAVIX (75 mg daily) to aspirin (325 mg daily). The patients randomized had: 1) recent histories of myocardial infarction (within 35 days); 2) recent histories of ischemic stroke (within 6 months) with at least a week of residual neurological signs; or 3) objectively established peripheral arterial disease. Patients received randomized treatment for an average of 1.6 years (maximum of 3 years).

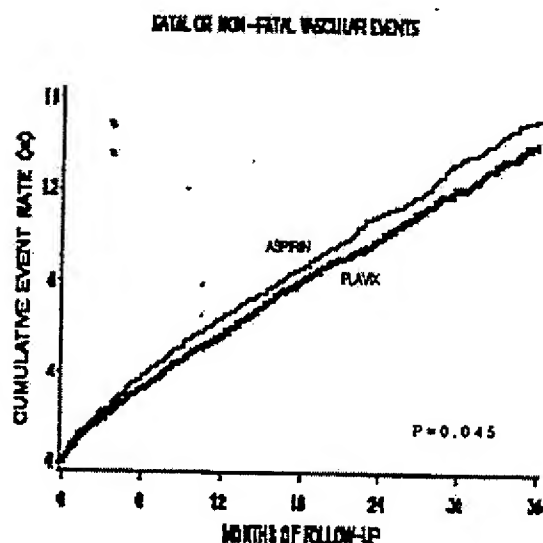
The trial's primary outcome was the time to first occurrence of new ischemic stroke (fatal or not), new myocardial infarction (fatal or not), or other vascular death. Deaths not easily attributable to nonvascular causes were all classified as vascular.

Outcome Events of the Primary Analysis

	PLAVIX	aspirin
Patients	9599	9586
IS (fatal or not)	438 (4.56%)	461 (4.81%)
MI (fatal or not)	275 (2.86%)	333 (3.47%)
Other vascular death	226 (2.35%)	226 (2.36%)
Total	939 (9.78%)	1020 (10.64%)

As shown in the table, PLAVIX was associated with a lower incidence of outcome events of every kind. The overall risk reduction (9.78% vs. 10.64%) was 8.7%, $P=0.045$. Similar results were obtained when all-cause mortality and all-cause strokes were counted instead of vascular mortality and ischemic strokes (risk reduction 6.9%). In patients who survived an on-study stroke or myocardial infarction, the incidence of subsequent events was again lower in the PLAVIX group.

The curves showing the overall event rate are shown in the figure. The event curves separated early and continued to diverge over the 3-year follow-up period.



Although the statistical significance favoring PLAVIX over aspirin was marginal ($P=0.045$), and represents the result of a single trial that has not been replicated, the comparator drug, aspirin, is itself effective (vs. placebo) in reducing cardiovascular events in patients with recent myocardial infarction or stroke. Thus, the difference between PLAVIX and placebo, although not measured directly, is substantial.

The CAPRIE trial included a population that was randomized on the basis of 3 entry criteria. The efficacy of PLAVIX relative to aspirin was heterogeneous across these randomized subgroups ($P=0.043$). It is not clear whether this difference is real or a chance occurrence. Although the CAPRIE trial was not designed to evaluate the relative benefit of PLAVIX over aspirin in the individual patient subgroups, the benefit appeared to be strongest in patients who were enrolled because of peripheral vascular disease (especially those who also had a history of myocardial infarction) and weaker in stroke patients. In patients who were enrolled in the trial on the sole basis of a recent myocardial infarction, PLAVIX was not numerically superior to aspirin.

In the meta-analyses of studies of aspirin vs. placebo in patients similar to those in CAPRIE, aspirin was associated with a reduced incidence of atherothrombotic events. There was a suggestion of heterogeneity in these studies too, with the effect strongest in patients with a history of myocardial infarction, weaker in patients with a history of stroke, and not discernible in patients with a history of peripheral vascular disease. With respect to the inferred comparison of PLAVIX to placebo, there is no indication of heterogeneity.

INDICATIONS AND USAGE

PLAVIX is indicated for the reduction of atherosclerotic events (myocardial infarction, stroke, and vascular death) in patients with atherosclerosis documented by recent stroke, recent myocardial infarction, or established peripheral arterial disease.

CONTRAINDICATIONS

The use of PLAVIX is contraindicated in the following conditions:

- i Hypersensitivity to the drug substance or any component of the product.
- ii Active pathological bleeding such as peptic ulcer or intracranial hemorrhage.

WARNINGS

None.

PRECAUTIONS

General

As with other anti-platelet agents, PLAVIX should be used with caution in patients who may be at risk of increased bleeding from trauma, surgery, or other pathological conditions. If a patient is to undergo elective surgery and an antiplatelet effect is not desired, PLAVIX should be discontinued 7 days prior to surgery.

GI Bleeding: PLAVIX prolongs the bleeding time. In CAPRIE, PLAVIX was associated with a rate of gastrointestinal bleeding of 2.0%, vs. 2.7% on aspirin. PLAVIX should be used with caution in patients who have lesions with a propensity to bleed (such as ulcers). Drugs that might induce such lesions (such as aspirin and other nonsteroidal anti-inflammatory drugs [NSAIDs]) should be used with caution in patients taking PLAVIX.

Use in Hepatically Impaired Patients:

Experience is limited in patients with severe hepatic disease, who may have bleeding diatheses. PLAVIX should be used with caution in this population.

Information for Patients

Patients should be told that it may take them longer than usual to stop bleeding when they take PLAVIX, and that they should report any unusual bleeding to their physician. Patients should inform physicians and dentists that they are taking PLAVIX before any surgery is scheduled and before any new drug is taken.

Drug Interactions

Study of specific drug interactions yielded the following results:

Aspirin: Aspirin did not modify the clopidogrel-mediated inhibition of ADP-induced platelet aggregation. Concomitant administration of 500 mg of aspirin twice a day for 1 day did not significantly increase the prolongation of bleeding time induced by PLAVIX. PLAVIX potentiated the effect of aspirin on collagen-induced platelet aggregation. The safety of chronic concomitant administration of aspirin and PLAVIX has not been established.

Heparin: In a study in healthy volunteers, PLAVIX did not necessitate modification of the heparin dose or alter the effect of heparin on coagulation. Coadministration of heparin had no effect on inhibition of platelet aggregation induced by PLAVIX. The safety of this combination has not been established, however, and concomitant use should be undertaken with caution.

Nonsteroidal Anti-Inflammatory Drugs (NSAIDs): In healthy volunteers receiving naproxen, concomitant administration of PLAVIX was associated with increased occult gastrointestinal blood loss. NSAIDs and PLAVIX should be coadministered with caution.

Warfarin: The safety of the coadministration of PLAVIX with warfarin has not been established. Consequently, concomitant administration of these two agents should be undertaken with caution. (See Precautions - General).

Other Concomitant Therapy: No clinically significant pharmacodynamic interactions were observed when PLAVIX was coadministered with atenolol, nifedipine, or both atenolol and nifedipine. The pharmacodynamic activity of PLAVIX was also not significantly influenced by the coadministration of phenobarbital, cimetidine or estrogen.

The pharmacokinetics of digoxin or theophylline were not modified by the coadministration of PLAVIX.

At high concentrations *in vitro*, clopidogrel inhibits P_{450} (2C9). Accordingly, PLAVIX may interfere with the metabolism of phenytoin, tamoxifen, tolbutamide, warfarin, torsemide, fluvastatin, and many non-steroidal anti-inflammatory agents, but there are no data with which to predict the magnitude of these interactions. Caution should be used when any of these drugs is coadministered with PLAVIX.

In addition to the above specific interaction studies, patients entered into CAPRIE received a variety of concomitant medications including diuretics, beta-blocking agents, angiotensin converting enzyme inhibitors, calcium antagonists, cholesterol lowering agents, coronary vasodilators, antidiabetic agents, antiepileptic agents and hormone replacement therapy without evidence of clinically significant adverse interactions.

Drug/Laboratory Test Interactions

None known.

Carcinogenesis, Mutagenesis, Impairment of Fertility

There was no evidence of tumorigenicity when clopidogrel was administered for 78 weeks to mice and 104 weeks to rats at dosages up to 77 mg/kg per day, which afforded plasma exposures >25 times that in humans at the recommended daily dose of 75 mg.

Clopidogrel was not genotoxic in four *in vitro* tests (Ames test, DNA-repair test in rat hepatocytes, gene mutation assay in Chinese hamster fibroblasts, and metaphase chromosome analysis of human lymphocytes) and in one *in vivo* test (micronucleus test by oral route in mice).

Clopidogrel was found to have no effect on fertility of male and female rats at oral doses up to 400 mg/kg per day (52 times the recommended human dose on a mg/m² basis).

Pregnancy

Pregnancy Category B. Reproduction studies performed in rats and rabbits at doses up to 500 and 300 mg/kg/day (respectively, 65 and 78 times the recommended daily human dose on a mg/m² basis), revealed no evidence of impaired fertility or fetotoxicity due to clopidogrel. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of a human response, PLAVIX should be used during pregnancy only if clearly needed.

Nursing Mothers

Studies in rats have shown that clopidogrel and/or its metabolites are excreted in the milk. It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the nursing woman.

Pediatric Use

Safety and effectiveness in the pediatric population have not been established.

ADVERSE REACTIONS

PLAVIX has been evaluated for safety in more than 11,300 patients, including over 7,000 patients treated for 1 year or more. The overall tolerability of PLAVIX was similar to that of aspirin regardless of age, gender and race, with an approximately equal incidence (13%) of patients withdrawing from treatment because of adverse reactions. The clinically important adverse events observed in CAPRIE are discussed below.

Hemorrhagic: In patients receiving PLAVIX in CAPRIE, gastrointestinal hemorrhage occurred at a rate of 2.0%, and required hospitalization in 0.7%. In patients receiving aspirin, the corresponding rates were 2.7% and 1.1%, respectively. The incidence of intracranial hemorrhage was 0.4% for PLAVIX compared to 0.5% for aspirin.

Neutropenia/agranulocytosis: Ticlopidine, a drug chemically similar to PLAVIX, is associated with a 0.8% rate of severe neutropenia (less than 450 neutrophils/ μ L). Patients in CAPRIE (see Clinical Trials) were intensively monitored for neutropenia. Severe neutropenia was observed in six patients, four on PLAVIX and two on aspirin. Two of the 9599 patients who received PLAVIX and none of the 9586 patients who received aspirin had neutrophil counts of zero.

One of the four PLAVIX patients was receiving cytotoxic chemotherapy, and another recovered and returned to the trial after only temporarily interrupting treatment with PLAVIX.

Although the risk of myelotoxicity with PLAVIX thus appears to be quite low, this possibility should be considered when a patient receiving PLAVIX demonstrates fever or other sign of infection.

Gastrointestinal: Overall, the incidence of gastrointestinal events (e.g. abdominal pain, dyspepsia, gastritis and constipation) in patients receiving PLAVIX was 27.1%, compared to 29.8% in those receiving aspirin.

The incidence of peptic, gastric or duodenal ulcers was 0.7% for PLAVIX and 1.2% for aspirin.

Cases of diarrhea were reported in 4.5% of patients in the PLAVIX group compared to 3.4% in the aspirin group. However, these were rarely severe (PLAVIX=0.2% and aspirin= 0.1%).

The incidence of patients withdrawing from treatment because of gastrointestinal adverse reactions was 3.2% for PLAVIX and 4.0% for aspirin.

Rash and Other Skin Disorders: The incidence of skin and appendage disorders in patients receiving PLAVIX was 15.8% (0.7% serious); the corresponding rate in aspirin patients was 13.1% (0.5% serious).

The overall incidence of patients withdrawing from treatment because of skin and appendage disorders adverse reactions was 1.5% for PLAVIX and 0.8% for aspirin.

Adverse events occurring in $\geq 2.5\%$ of patients on PLAVIX in the CAPRIE controlled clinical trial are shown below regardless of relationship to PLAVIX. The median duration of therapy was 20 months, with a maximum of 3 years.

**Adverse Events Occurring in $\geq 2.5\%$
of PLAVIX Patients**

<i>Body System Event</i>	<i>% Incidence (% Discontinuation)</i>	
	<i>PLAVIX [n=9599]</i>	<i>Aspirin [n=9586]</i>
<i>Body as a Whole - general disorders</i>		
Chest Pain	8.3 (0.2)	8.3 (0.3)
Accidental Injury	7.9 (0.1)	7.3 (0.1)
Influenza-like symptoms	7.5 (<0.1)	7.0 (<0.1)
Pain	6.4 (0.1)	6.3 (0.1)
Fatigue	3.3 (0.1)	3.4 (0.1)
<i>Cardiovascular disorders, general</i>		
Edema	4.1 (<0.1)	4.5 (<0.1)
Hypertension	4.3 (<0.1)	5.1 (<0.1)
<i>Central & peripheral nervous system disorders</i>		
Headache	7.6 (0.3)	7.2 (0.2)
Dizziness	6.2 (0.2)	6.7 (0.3)
<i>Gastrointestinal system disorders</i>		
Abdominal pain	5.6 (0.7)	7.1 (1.0)
Dyspepsia	5.2 (0.6)	6.1 (0.7)
Diarrhea	4.5 (0.4)	3.4 (0.3)
Nausea	3.4 (0.5)	3.8 (0.4)
<i>Metabolic & nutritional disorders</i>		
Hypercholesterolemia	4.0 (0)	4.4 (<0.1)
<i>Musculo-skeletal system disorders</i>		
Arthralgia	6.3 (0.1)	6.2 (0.1)
Back Pain	5.8 (0.1)	5.3 (<0.1)
<i>Platelet, bleeding, & clotting disorders</i>		
Purpura	5.3 (0.3)	3.7 (0.1)
Epistaxis	2.9 (0.2)	2.5 (0.1)
<i>Psychiatric disorders</i>		
Depression	3.6 (0.1)	3.9 (0.2)
<i>Respiratory system disorders</i>		
Upper resp tract infection	8.7 (<0.1)	8.3 (<0.1)
Dyspnea	4.5 (0.1)	4.7 (0.1)
Rhinitis	4.2 (0.1)	4.2 (<0.1)
Bronchitis	3.7 (0.1)	3.7 (0)
Coughing	3.1 (<0.1)	2.7 (<0.1)
<i>Skin & appendage disorders</i>		
Rash	4.2 (0.5)	3.5 (0.2)
Pruritus	3.3 (0.3)	1.6 (0.1)
<i>Urinary system disorders</i>		
Urinary tract infection	3.1 (0)	3.5 (0.1)

Incidence of discontinuation, regardless of relationship to therapy, is shown in parentheses.

Other adverse experiences of potential importance occurring in 1% to 2.5% of patients receiving PLAVIX in the CAPRIE controlled clinical trial are listed below regardless of relationship to PLAVIX. In general, the incidence of these events was similar in the aspirin-treated group.

Autonomic Nervous System Disorders: Syncope, Palpitation. *Body as a Whole - general disorders:* Asthenia, Hernia. *Cardiovascular disorders:* Cardiac failure. *Central and peripheral nervous system disorders:* Cramps legs, Hypoaesthesia, Neuralgia, Paraesthesia, Vertigo. *Gastro-intestinal system disorders:* Constipation, Vomiting. *Heart rate and rhythm disorders:* Fibrillation atrial. *Liver and biliary system disorders:* Hepatic enzymes increased. *Metabolic and nutritional disorders:* Gout, hyperuricemia, non-protein nitrogen (NPN) increased. *Musculo-skeletal system disorders:* Arthritis, Arthrosis. *Platelet, bleeding & clotting disorders:* GI hemorrhage, hematoma, platelets decreased. *Psychiatric disorders:* Anxiety, Insomnia. *Red blood cell disorders:* Anemia. *Respiratory system disorders:* Pneumonia, Sinusitis. *Skin and appendage disorders:* Eczema, Skin ulceration. *Urinary system disorders:* Cystitis. *Vision disorders:* Cataract, Conjunctivitis.

Other potentially serious adverse events which may be of clinical interest but were rarely reported (<1%) in patients who received PLAVIX are listed below regardless of relationship to PLAVIX. In general, the incidence of these events was similar in the aspirin group.

Body as a whole: Allergic reaction, necrosis ischemic. *Cardiovascular disorders:* Edema generalized. *Gastrointestinal system disorders:* Gastric ulcer perforated, gastritis hemorrhagic, upper GI ulcer hemorrhagic. *Liver and Biliary system disorders:* Bilirubinemia, hepatitis infectious, liver fatty. *Platelet, bleeding and clotting disorders:* hemarthrosis, hematuria, hemoptysis, hemorrhage intracranial, hemorrhage retroperitoneal, hemorrhage of operative wound, ocular hemorrhage, pulmonary hemorrhage, purpura allergic, thrombocytopenia. *Red blood cell disorders:* Anemia aplastic, anemia hypochromic. *Reproductive disorders, female:* Menorrhagia. *Respiratory system disorders:* Hemothorax. *Skin and appendage disorders:* Bullous eruption, rash erythematous, rash maculopapular, urticaria. *White cell and reticuloendothelial system disorders:* Agranulocytosis, granulocytopenia, leukemia, leukopenia, neutrophils decreased.

OVERDOSAGE

One case of deliberate overdosage with PLAVIX was reported in the large, controlled clinical study. A 34-year-old woman took a single 1,050-mg dose of PLAVIX (equivalent to 14 standard 75-mg tablets). There were no associated adverse events. No special therapy was instituted, and she recovered without sequelae.

No adverse events were reported after single oral administration of 600 mg (equivalent to 8 standard 75-mg tablets) of PLAVIX in healthy volunteers. The bleeding time was prolonged by a factor of 1.7, which is similar to that typically observed with the therapeutic dose of 75 mg of PLAVIX per day.

A single oral dose of clopidogrel at 1500 or 2000 mg/kg was lethal to mice and to rats and at 3000 mg/kg to baboons. Symptoms of acute toxicity were vomiting (in baboons), prostration, difficult breathing, and gastrointestinal hemorrhage in all species.

Recommendations About Specific Treatment:
Based on biological plausibility, platelet transfusion may be appropriate to reverse the pharmacological effects of PLAVIX if quick reversal is required.

DOSAGE AND ADMINISTRATION

The recommended dose of PLAVIX is 75 mg once daily with or without food.

No dosage adjustment is necessary for elderly patients or patients with renal disease. (See Clinical Pharmacology: Special Populations.)

HOW SUPPLIED

PLAVIX is available as a pink, round, biconvex, film-coated tablet engraved with "75" on one side. Tablets are provided as follows:

NDC 63653-1171-4 bottles of 100
NDC 63653-1171-5 bottles of 500
NDC 63653-1171-3 blisters of 100

Storage

Store at 25°C (77°F); excursions permitted to 15°-30°C (59°-86°F) [see USP Controlled Room Temperature]

Caution: Federal law prohibits dispensing without a prescription.

Manufactured by:
Sanofi Pharmaceuticals, Inc.
New York, NY 10016

Distributed by:
Bristol-Myers Squibb/
Sanofi Pharmaceuticals
Partnership
New York, NY 10016

PLAVIX® is a registered trademark of Sanofi

Date of Labeling Approval

Exhibit 16



CPMP/854/98

**COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS
EUROPEAN PUBLIC ASSESSMENT REPORT (EPAR)**

PLAVIX

International Nonproprietary Name (INN): **Clopidogrel**

Abstract

On 15 July 1998, the European Commission issued a Marketing Authorisation valid throughout the European Union for the medicinal product Plavix, which contains clopidogrel. This decision was based on the assessment report and the favourable opinion adopted by the Committee for Proprietary Medicinal Products (CPMP) on 25 March 1998. The Marketing Authorisation Holder responsible for this medicinal product is Sanofi Pharma Bristol-Myers Squibb SNC.

Clopidogrel, the active ingredient in Plavix tablets, belongs to a group of medicines called antiplatelet medicinal products. Platelets are very small structures, smaller than red or white blood cells, which clump together during blood clotting. By preventing this clumping, antiplatelet medicinal products reduce the chances of blood clots forming.

Plavix is indicated for the reduction of atherosclerotic events (myocardial infarction, stroke, death due to vascular causes) in patients with a history of symptomatic atherosclerotic disease defined by ischaemic stroke (from 7 days until less than 6 months), myocardial infarction (from a few days until less than 35 days) or established peripheral arterial disease.

Detailed conditions for the use of this product are described in the Summary of Product Characteristics (SPC) which can be found in this EPAR and is available in all European Union official languages.

This indication is based on the results of one comparative international multicentre clinical trial (the "CAPRIE" study, Clopidogrel versus acetyl salicylic acid in patients at risk on ischaemic events) involving 19185 patients, comparing clopidogrel with acetyl salicylic acid (ASA). The results showed that clopidogrel at a dose of 75 mg once daily significantly reduced the incidence of new ischaemic events compared to acetyl salicylic acid (325 mg once daily). The slight but statistically significant difference of clopidogrel over acetyl salicylic acid was mainly related to patients enrolled due to peripheral arterial disease.

Clopidogrel was well tolerated, having a safety profile comparable to ASA, but with a better gastrointestinal tolerability. Only rash, purpura and diarrhoea were reported with a higher frequency with clopidogrel but were rarely severe.

The CPMP, on the basis of efficacy and safety data submitted considered that Plavix showed adequate evidence of efficacy and a satisfactory safety profile, and therefore recommended that the Marketing Authorisation should be granted.

<u>EU Number</u>	<u>Name</u>	<u>Strength</u>	<u>Pharmaceutical Form</u>	<u>Route of administration</u>	<u>Packaging</u>	<u>Package size</u>
EU/1/98/069/001a	Plavix	75 mg	Film-coated tablet	Oral use	Blister (PVC/PVDC)	28
EU/1/98/069/001b	Plavix	75 mg	Film-coated tablet	Oral use	Blister (Aluminium)	28
EU/1/98/069/002a	Plavix	75 mg	Film-coated tablet	Oral use	Blister (PVC/PVDC)	50
EU/1/98/069/002b	Plavix	75 mg	Film-coated tablet	Oral use	Blister (Aluminium)	50
EU/1/98/069/003a	Plavix	75 mg	Film-coated tablet	Oral use	Blister (PVC/PVDC)	84
EU/1/98/069/003b	Plavix	75 mg	Film-coated tablet	Oral use	Blister (Aluminium)	84

PACKAGE LEAFLET

**PLAVIX 75 MG TABLETS
(CLOPIDOGREL)**

Please read this leaflet carefully. It contains important information about your medicine and your medical condition. If you have any questions after reading the leaflet, please talk to your doctor or pharmacist.

The name of this medicine is Plavix.

What does Plavix contain?

Each tablet of Plavix tablets contains 75 milligrams (mg) of the active substance called clopidogrel.

What else do Plavix tablets contain?

As well as the active substance in Plavix (clopidogrel), the tablets contain a number of other ingredients. Some people may be sensitive or allergic to one or more of these ingredients, which are: maize (corn) starch, lactose (milk sugar), castor oil, cellulose, macrogol 6000, iron oxide (E172), titanium dioxide (E171), hypromellose, and carnauba wax.

What do Plavix tablets look like?

Plavix tablets are round, pink, engraved on one side with the number «75» and on the other side with the number «1171». They are supplied in cardboard cartons containing 28, 50 and 84 tablets in PVC/PVDC blisters or in all aluminium blisters.

What type of medicine is Plavix?

Clopidogrel, the active ingredient in Plavix tablets, belongs to a group of medicines called antiplatelet medicinal products. Platelets are very small structures, smaller than red or white blood cells, which clump together during blood clotting. By preventing this clumping, antiplatelet medicinal products reduce the chances of blood clots forming (a process called thrombosis).

Who is responsible for marketing Plavix?

Sanofi Pharma Bristol-Myers Squibb SNC
174 Avenue de France - 75013 Paris - France

Who manufactures Plavix?

Plavix is manufactured by:
Sanofi Winthrop Industrie 1, rue de la Vierge, 33440 Ambarès, France
and
Sanofi Winthrop Ltd, Production Division, Edgefield Avenue, Fawdon
Newcastle Upon Tyne NE3 3TT, United Kingdom

What is Plavix used for?

You have been prescribed Plavix because you have a condition known as hardening of the arteries (also known as atherosclerosis). Atherosclerosis results in a narrowing of the blood vessels (arteries) and an increased risk of blood clots (thrombi). This can lead to a stroke or a heart attack. Plavix is taken to prevent blood clots forming in the hardened arteries (a process known as atherothrombosis), thus reducing the risk of a stroke or a heart attack.

This product has been prescribed for you personally and you should not pass it on to others.

Who should NOT take Plavix ?

You should not take Plavix:

- If you have had a bad reaction (allergy) in the past to any of the substances contained in the tablets. Please make sure you read «What do Plavix tablets contain?» and «What else do Plavix tablets contain?»

- If you have a medical condition that is causing bleeding such as a stomach ulcer.
- If you suffer from severe liver disease.
- If you are breast-feeding.

If you think you may have any of these problems, or if you are in any doubt at all, consult your doctor before taking Plavix.

What needs to be considered when taking Plavix?

If any of the following situations apply to you, you should tell your doctor at once:

- You have had a recent serious injury
- You have recently undergone surgery (including dental)
- You have a blood disorder that makes you prone to internal bleeding (bleeding inside any tissues, organs or joints of your body).
- You have a medical condition that puts you at risk of internal bleeding (such as a stomach ulcer)
- You will be having surgery (including dental) in the next two weeks
- You are taking another type of medication. This includes all medications, even those which you have purchased yourself without a medical prescription.
- You have kidney or liver disease

What if you experience prolonged bleeding when taking Plavix?

If you cut or injure yourself, it may take slightly longer than usual for bleeding to stop. This is linked to the way your medicine works. For minor cuts and injuries e.g. cutting yourself shaving, this is of no concern. However, if you are in any doubt at all, you should contact your doctor straightaway.

What if you take other medicines while you are taking Plavix?

Some other medicines, whether prescribed by your doctor or bought over the pharmacy counter, may interact with the actions of Plavix to have unwanted effects.

If you are in any doubt about whether you should take another medicine while taking Plavix, please see your doctor or pharmacist.

Other medicines that are not recommended with Plavix:

- Aspirin (acetylsalicylic acid), when taken for prolonged periods, except when it has been specifically recommended by your doctor. An occasional dose of aspirin (no more than 1000 mg in any 24 hour period) should not cause a problem.
- Other medicinal products used to reduce blood clotting such as warfarin and heparin.
- Non-Steroidal Anti-Inflammatory Medicinal products (medicinal products used to treat painful and/or inflammatory conditions of muscles or joints) when taken for prolonged periods.

What if you are pregnant or breast-feeding?

If you are pregnant or if you are a mother breast-feeding a baby, you should tell your doctor before taking Plavix. If you become pregnant while taking Plavix, consult your doctor straightaway.

Will Plavix have any effects on your ability to drive or operate machinery?

Your ability to drive or to operate complicated machinery should not be affected by Plavix.

How should Plavix be taken?

Adults (including the elderly):

You should take one 75 mg tablet of Plavix per day, with or without food. You should take your medicine regularly and at the same time each day.

Children and adolescents:

Plavix is not intended for children or adolescents below the age of 18 years.

How long should you continue to take Plavix?

You should take Plavix for as long as your doctor continues to prescribe it.

What if you take too many Plavix tablets at once?

If you take an overdose of tablets inform your doctor at once or go to the nearest hospital emergency department. A large dose of tablets could put you at risk of serious bleeding, requiring emergency treatment.

What if you miss a dose of Plavix?

If you forget to take a dose of Plavix, but remember within 12 hours of your usual time, take your tablet straightaway and then take your next tablet at the normal time. If you forget for more than 12 hours simply take the next single dose at the usual time. Do not take a double dose to make up for the one you missed. You can check the day on which you last took a tablet of Plavix by referring to the calendar printed on the blister.

What undesirable effects may Plavix cause?

Occasional side-effects reported with Plavix are:

- Skin disorders such as rashes and/or itching
- Diarrhoea
- Abdominal pain
- Indigestion or heartburn
- Constipation
- Nausea
- Vomiting
- Dizziness
- Headache
- Tingling sensation in hands and feet
- Bleeding in the stomach or bowels
- Nose bleeds
- Bruising
- Blood in the urine
- Hepatic and biliary disorders
- Generalised allergic reactions such as swelling of the face, lips and/or tongue, shortness of breath.

Bleeding from blood vessels in the eye and inside the head has been reported in a small number of cases.

If you notice any undesirable effects, including any not mentioned above, tell your doctor or pharmacist.

How long should you keep your Plavix tablets?

Do not use your tablets after the expiry date stated on the carton and on the blister.

How should your Plavix tablets be stored?

Plavix tablets should be stored in a safe place and kept out of the reach of children. Do not leave them near a radiator, on a window sill or in a humid place. Do not remove the tablets from the blister pack until you are ready to take your medicine.

Further information:

For any further information about Plavix, you can contact the local representative of the marketing authorisation holder. The name and address of the local representative in each country of the European Union is given below:

Belgique/België/Belgien

SANOFI-SYNTHELABO S.A. N.V.
Avenue de la Métrologie, 5 /
Metrologielaan, 5
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Tel. : +32 2 244 10 00

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Deutschland

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SANOFI-SYNTHELABO

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France

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SUMMARY OF PRODUCT CHARACTERISTICS

1. NAME OF THE MEDICINAL PRODUCT

Plavix 75 mg film-coated tablets

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

Clopidogrel hydrogen sulphate 97.875 mg (molar equivalent of 75 mg of clopidogrel base)

3. PHARMACEUTICAL FORM

Film-coated tablet.

Plavix 75 mg film-coated tablets are pink, round, biconvex, film-coated, engraved with « 75 » on one side and « 1171 » on the other side.

4. CLINICAL PARTICULARS

4.1 Therapeutic indications

Reduction of atherosclerotic events (myocardial infarction, stroke, death due to vascular causes) in patients with a history of symptomatic atherosclerotic disease defined by ischaemic stroke (from 7 days until less than 6 months), myocardial infarction (from a few days until less than 35 days) or established peripheral arterial disease.

This indication is based on the results of the CAPRIE study comparing clopidogrel with acetyl salicylic acid (ASA). The slight but statistically significant difference of clopidogrel over ASA was mainly related to patients enrolled due to peripheral arterial disease.

For further information please refer to section 4.4 Special warnings and special precautions for use and 5.1 Pharmacodynamic properties.

4.2 Posology and method of administration

- Adults and elderly

Clopidogrel should be given as a single daily dose of 75 mg with or without food.

- Children and adolescents

Safety and efficacy in subjects below the age of 18 have not been established.

4.3 Contra-indications

Hypersensitivity to the active substance or any component of the medicinal product.

Severe liver impairment.

Active pathological bleeding such as peptic ulcer or intracranial haemorrhage.

Breast-feeding (see 4.6 Use during pregnancy and lactation).

4.4 Special warnings and special precautions for use

In patients with acute myocardial infarction, clopidogrel therapy should not be initiated within the first few days following myocardial infarction.

In view of the lack of data, clopidogrel cannot be recommended in unstable angina, PTCA (stenting), CABG and acute ischaemic stroke (less than 7 days).

As with other anti-platelet agents, clopidogrel should be used with caution in patients who may be at risk of increased bleeding from trauma, surgery or other pathological conditions. If a patient is to undergo elective surgery and an antiplatelet effect is not desired, clopidogrel should be discontinued 7 days prior to surgery.

Clopidogrel prolongs bleeding time and should be used with caution in patients who have lesions with a propensity to bleed (particularly gastrointestinal and intraocular).

Patients should be told that it may take longer than usual to stop bleeding when they take clopidogrel, and that they should report any unusual bleeding to their physician. Patients should inform physicians and dentists that they are taking clopidogrel before any surgery is scheduled and before any new drug is taken.

Therapeutic experience with clopidogrel is limited in patients with renal impairment. Therefore clopidogrel should be used with caution in these patients.

Experience is limited in patients with moderate hepatic disease who may have bleeding diatheses. Clopidogrel should therefore be used with caution in this population.

The concomitant administration of clopidogrel with warfarin is not recommended since it may increase the intensity of bleedings.

In view of the possible increased risk of bleeding, the concomitant administration of clopidogrel with ASA, non-steroidal anti-inflammatory drugs, heparin, or thrombolytics should be undertaken with caution (see 4.5 Interaction with other medicinal products and other forms of interaction).

Drugs that might induce gastrointestinal lesions (such as Non-Steroidal Anti-Inflammatory Drugs) should be used with caution in patients taking clopidogrel (see 4.5 Interaction with other medicinal products and other forms of interaction).

4.5 Interaction with other medicinal products and other forms of interaction

Warfarin: see 4.4 Special warnings and special precautions for use.

Acetylsalicylic acid (ASA): ASA did not modify the clopidogrel-mediated inhibition of ADP-induced platelet aggregation, but clopidogrel potentiated the effect of ASA on collagen-induced platelet aggregation. However, concomitant administration of 500 mg of ASA twice a day for one day did not significantly increase the prolongation of bleeding time induced by clopidogrel intake. The safety of the chronic concomitant administration of ASA and clopidogrel has not been established (see 4.4 Special warnings and special precautions for use).

Heparin: in a clinical study conducted in healthy subjects, clopidogrel did not necessitate modification of the heparin dose or alter the effect of heparin on coagulation. Co-administration of heparin had no effect on the inhibition of platelet aggregation induced by clopidogrel. However, the safety of this combination has not been established and concomitant use should be undertaken with caution (see 4.4 Special warnings and special precautions for use).

Thrombolytics: the safety of the concomitant administration of clopidogrel, rt-PA and heparin was assessed in patients with recent myocardial infarction. The incidence of clinically significant bleeding was similar to that observed when rt-PA and heparin are co-administered with ASA. The safety of the concomitant administration of clopidogrel with other thrombolytic agents has not been established and should be undertaken with caution (see 4.4 Special warnings and special precautions for use).

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs): in a clinical study conducted in healthy volunteers, the concomitant administration of clopidogrel and naproxen increased occult gastrointestinal blood loss. However, due to the lack of interaction studies with other NSAIDs it is presently unclear whether there is an increased risk of gastrointestinal bleeding with all NSAIDs. Consequently, NSAIDs and clopidogrel should be co-administered with caution (see 4.4 Special warnings and special precautions for use).

Other concomitant therapy: a number of other clinical studies have been conducted with clopidogrel and other concomitant medications to investigate the potential for pharmacodynamic and pharmacokinetic interactions. No clinically significant pharmacodynamic interactions were observed when clopidogrel was co-administered with atenolol, nifedipine, or both atenolol and nifedipine. Furthermore, the pharmacodynamic activity of clopidogrel was not significantly influenced by the co-administration of phenobarbital, cimetidine, or oestrogen.

The pharmacokinetics of digoxin or theophylline were not modified by the co-administration of clopidogrel. Antacids did not modify the extent of clopidogrel absorption.

Data from studies with human liver microsomes indicated that the carboxylic acid metabolite of clopidogrel could inhibit the activity of Cytochrome P450 2C9. This could potentially lead to increased plasma levels of drugs such as phenytoin and tolbutamide and the NSAIDs which are metabolised by Cytochrome P450 2C9. Data from the CAPRIE study indicate that phenytoin and tolbutamide can be safely coadministered with clopidogrel.

4.6 Use during pregnancy and lactation

- **Pregnancy**

Reproduction studies performed in rats and in rabbits revealed no evidence of impaired fertility or harm to the foetus due to clopidogrel. There are, however, no adequate and well-controlled studies in pregnant women. In view of the lack of data, clopidogrel is not recommended during pregnancy.

- **Lactation**

Studies in rats have shown that clopidogrel and/or its metabolites are excreted in the milk. It is not known whether this medicinal product is excreted in human milk (see 4.3 Contra-indications).

4.7 Effects on ability to drive and use machines

No impairment of driving or psychometric performance was observed following clopidogrel administration.

4.8 Undesirable effects

Clopidogrel has been evaluated for safety in more than 11,300 patients, including over 7,000 patients treated for 1 year or more. Clopidogrel 75 mg/day was well tolerated compared to ASA 325 mg/day in a large controlled clinical trial (CAPRIE). The overall tolerability of clopidogrel in this study was similar

to ASA, regardless of age, gender and race. The clinically relevant adverse effects observed in CAPRIE are discussed below.

Haemorrhagic disorders: in patients treated with either clopidogrel or ASA, the overall incidence of any bleeding was 9.3%. The incidence of severe cases was 1.4% for clopidogrel and 1.6% for ASA. In patients that received clopidogrel, gastrointestinal bleeding occurred at a rate of 2.0%, and required hospitalisation in 0.7%. In patients that received ASA, the corresponding rates were 2.7% and 1.1%, respectively.

The incidence of other bleeding was higher in patients that received clopidogrel compared to ASA (7.3% vs. 6.5%). However, the incidence of severe events was similar in both treatment groups (0.6% vs. 0.4%). The most frequently reported events in both treatment groups were : purpura/bruising/haematoma, and epistaxis. Other less frequently reported events were haematoma, haematuria, and eye bleeding (mainly conjunctival).

The incidence of intracranial bleeding was 0.4% in patients that received clopidogrel and 0.5% for patients that received ASA .

Haematological: severe neutropaenia ($<0.45 \times 10^9/l$) was observed in 4 patients (0.04%) that received clopidogrel and 2 patients (0.02%) that received ASA. Two of the 9599 patients who received clopidogrel and none of the 9586 patients who received ASA had neutrophil counts of zero. One case of aplastic anaemia occurred on clopidogrel treatment.

The incidence of severe thrombocytopaenia ($<80 \times 10^9/l$) was 0.2% on clopidogrel and 0.1% on ASA ; very rare cases of platelet count $\leq 30 \times 10^9/l$ have been reported.

Gastrointestinal: the overall, incidence of gastrointestinal events (e.g. abdominal pain, dyspepsia, gastritis and constipation) was significantly lower in patients treated with clopidogrel compared to ASA (27.1% vs. 29.8%). In addition, the number of events resulting in early permanent discontinuation was lower in the clopidogrel group compared to ASA (3.2% vs. 4.0%). However, the incidence of adverse events judged as clinically severe were not statistically different in the groups (3.0% vs. 3.6%). The most frequently reported events in both treatment groups were: abdominal pain, dyspepsia, diarrhoea, and nausea. Other less frequently reported events were constipation, tooth disorder, vomiting, flatulence and gastritis.

Cases of diarrhoea were reported at a significantly higher frequency in patients taking clopidogrel compared to ASA (4.5% vs. 3.4%). The incidence of severe diarrhoea was similar in both treatment groups (0.2% vs. 0.1%). The incidence of peptic, gastric or duodenal ulcers was 0.7% for clopidogrel and 1.2% for ASA.

Skin and appendage disorders: the overall incidence of skin and appendage disorders in patients taking clopidogrel was significantly higher (15.8%) compared to ASA (13.1%). The incidence of severe events was similar in both treatment groups (0.7% vs. 0.5%).

There were significantly more patients with rash in the clopidogrel group compared to the ASA group (4.2% vs. 3.5%). More patients reported pruritus in the clopidogrel group compared to ASA (3.3% vs. 1.6%).

Central and peripheral nervous system disorders : the overall incidence of central and peripheral nervous system disorders (e.g. headache, dizziness, vertigo and paraesthesia) was significantly lower in patients taking clopidogrel compared to ASA (22.3% vs. 23.8%).

Hepatic and biliary disorders : the overall incidence of hepatic and biliary disorders was similar in patients treated with clopidogrel compared to ASA (3.5% vs. 3.4%).

Post-marketing experience : The post-marketing experience confirms the safety profile defined during the clinical development ; hypersensitivity reactions have been reported : these mainly include skin

reactions (maculopapular or erythematous rash, urticaria....) and/or pruritus. Very rare cases of bronchospasm, angioedema or anaphylactoid reactions have been observed. As part of the marketing experience, very rare cases of Thrombotic Thrombocytopenic Purpura (TTP) (1/200.000 exposed patients) have been reported.

4.9 Overdose

One case of deliberate overdosage with clopidogrel has been reported. A 34 year old woman took a single 1,050 mg dose of clopidogrel (equivalent to 14 standard 75 mg tablets). There were no associated undesirable effects. No special therapy was instituted and she recovered without sequelae. No adverse events were reported after single oral administration of 600 mg (equivalent to 8 standard 75 mg tablets) of clopidogrel to healthy subjects. The bleeding time was prolonged by a factor of 1.7 which is similar to that typically observed with the therapeutic dose of 75 mg per day.

No antidote to the pharmacological activity of clopidogrel has been found. If prompt correction of prolonged bleeding time is required, platelet transfusion may reverse the effects of clopidogrel.

5. PHARMACOLOGICAL PROPERTIES

5.1 Pharmacodynamic properties

Pharmacotherapeutical group: platelet aggregation inhibitors excl. Heparin, ATC Code: B01AC/04.

Clopidogrel selectively inhibits the binding of adenosine diphosphate (ADP) to its platelet receptor, and the subsequent ADP-mediated activation of the GPIIb/IIIa complex, thereby inhibiting platelet aggregation. Biotransformation of clopidogrel is necessary to produce inhibition of platelet aggregation. Clopidogrel also inhibits platelet aggregation induced by other agonists by blocking the amplification of platelet activation by released ADP. Clopidogrel acts by irreversibly modifying the platelet ADP receptor. Consequently, platelets exposed to clopidogrel are affected for the remainder of their lifespan and recovery of normal platelet function occurs at a rate consistent with platelet turnover.

Repeated doses of 75 mg per day produced substantial inhibition of ADP-induced platelet aggregation from the first day; this increased progressively and reached steady state between Day 3 and Day 7. At steady state, the average inhibition level observed with a dose of 75 mg per day was between 40% and 60%. Platelet aggregation and bleeding time gradually returned to baseline values, generally within 5 days after treatment was discontinued.

The safety and efficacy of clopidogrel in preventing vascular ischaemic events have been evaluated in a blinded comparison with ASA (CAPRIE, Clopidogrel versus ASA in Patients at Risk of Ischaemic Events). This study included 19,185 patients with atherothrombosis as manifested by recent myocardial infarction (<35 days), recent ischaemic stroke (between 7 days and 6 months) or established peripheral arterial disease (PAD). Patients were randomised to clopidogrel 75 mg/day or ASA 325 mg/day, and were followed for 1 to 3 years. In the myocardial infarction subgroup, most of the patients received ASA for the first few days following the acute myocardial infarction.

Clopidogrel significantly reduced the incidence of new ischaemic events (combined end point of myocardial infarction, ischaemic stroke and vascular death) when compared to ASA. In the intention to treat analysis, 939 events were observed in the clopidogrel group and 1,020 events with ASA (relative risk reduction (RRR) 8.7%, [95% CI: 0.2 to 16.4] ; $p = 0.045$), which corresponds, for every 1000 patients treated for 2 years, to 10 [CI: 0 to 20] additional patients being prevented from experiencing a new ischaemic event. Analysis of total mortality as a secondary endpoint did not show any significant difference between clopidogrel (5.8%) and ASA (6.0%).

In a subgroup analysis by qualifying condition (myocardial infarction, ischaemic stroke, and PAD) the benefit appeared to be strongest (achieving statistical significance at $p = 0.003$) in patients enrolled due

to PAD (especially those who also had a history of myocardial infarction) (RRR = 23.7% ; CI: 8.9 to 36.2) and weaker (not significantly different from ASA) in stroke patients (RRR = 7.3% ; CI: -5.7 to 18.7). In patients who were enrolled in the trial on the sole basis of a recent myocardial infarction, clopidogrel was numerically inferior, but not statistically different from ASA (RRR = -4.0% ; CI: -22.5 to 11.7). In addition, a subgroup analysis by age suggested that the benefit of clopidogrel in patients over 75 years was less than that observed in patients ≤ 75 years

Since the CAPRIE trial was not powered to evaluate efficacy of individual subgroups, it is not clear whether the differences in relative risk reduction across qualifying conditions are real, or a result of chance.

5.2 Pharmacokinetic properties

After repeated oral doses of 75 mg per day, clopidogrel is rapidly absorbed. However, plasma concentrations of the parent compound are very low and below the quantification limit (0.00025 mg/l) beyond 2 hours. Absorption is at least 50%, based on urinary excretion of clopidogrel metabolites.

Clopidogrel is extensively metabolised by the liver and the main metabolite, which is inactive, is the carboxylic acid derivative which represents about 85% of the circulating compound in plasma. Peak plasma levels of this metabolite (approx. 3mg/l after repeated 75 mg oral doses) occurred approximately 1 hour after dosing.

Clopidogrel is a prodrug. The active metabolite, a thiol derivative, is formed by oxidation of clopidogrel to 2-oxo-clopidogrel and subsequent hydrolysis. The oxidative step is regulated primarily by Cytochrome P₄₅₀ isoenzymes 2B6 and 3A4 and to a lesser extent by 1A1, 1A2 and 2C19. The active thiol metabolite, which has been isolated *in vitro*, binds rapidly and irreversibly to platelet receptors, thus inhibiting platelet aggregation. This metabolite has not been detected in plasma.

The kinetics of the main circulating metabolite were linear (plasma concentrations increased in proportion to dose) in the dose range of 50 to 150 mg of clopidogrel.

Clopidogrel and the main circulating metabolite bind reversibly *in vitro* to human plasma proteins (98% and 94% respectively). The binding is non-saturable *in vitro* over a wide concentration range.

Following an oral dose of ¹⁴C-labelled clopidogrel in man, approximately 50% was excreted in the urine and approximately 46% in the faeces in the 120 hour interval after dosing. The elimination half-life of the main circulating metabolite was 8 hours after single and repeated administration.

After repeated doses of 75 mg clopidogrel per day, plasma levels of the main circulating metabolite were lower in subjects with severe renal disease (creatinine clearance from 5 to 15 ml/min) compared to subjects with moderate renal disease (creatinine clearance from 30 to 60 ml/min) and to levels observed in other studies with healthy subjects. Although inhibition of ADP-induced platelet aggregation was lower (25%) than that observed in healthy subjects, the prolongation of bleeding was similar to that seen in healthy subjects receiving 75 mg of clopidogrel per day. In addition, clinical tolerance was good in all patients.

The pharmacokinetics and pharmacodynamics of clopidogrel were assessed in a single and multiple dose study in both healthy subjects and those with cirrhosis (Child-Pugh class A or B). Daily dosing for 10 days with clopidogrel 75 mg/day was safe and well tolerated. Clopidogrel C_{max} for both single dose and steady state for cirrhotics was many fold higher than in normal subjects. However, plasma levels of the main circulating metabolite together with the effect of clopidogrel on ADP-induced platelet aggregation and bleeding time were comparable between these groups.

5.3 Preclinical safety data

During preclinical studies in rat and baboon, the most frequently observed effects were liver changes. These occurred at doses representing at least 25 times the exposure seen in humans receiving the clinical dose of 75 mg/day and were a consequence of an effect on hepatic metabolising enzymes. No effect on hepatic metabolising enzymes were observed in humans receiving clopidogrel at the therapeutic dose. At very high doses, a poor gastric tolerability (gastritis, gastric erosions and/or vomiting) of clopidogrel was also reported in rat and baboon.

There was no evidence of carcinogenic effect when clopidogrel was administered for 78 weeks to mice and 104 weeks to rats when given at doses up to 77 mg/kg per day (representing at least 25 times the exposure seen in humans receiving the clinical dose of 75 mg/day).

Clopidogrel has been tested in a range of *in vitro* and *in vivo* genotoxicity studies, and showed no genotoxic activity.

Clopidogrel was found to have no effect on the fertility of male and female rats and was not teratogenic in either rats or rabbits. When given to lactating rats, clopidogrel caused a slight delay in the development of the offspring. Specific pharmacokinetic studies performed with radiolabelled clopidogrel have shown that the parent compound or its metabolites are excreted in the milk. Consequently, a direct effect (slight toxicity), or an indirect effect (low palatability) cannot be excluded.

6. PHARMACEUTICAL PARTICULARS

6.1 List of excipients

Core:

Anhydrous lactose

Modified maize starch

Macrogol 6000

Microcrystalline cellulose

Hydrogenated castor oil

Coating:

Hypromellose

Macrogol 6000

Titanium dioxide (E171)

Red iron oxide (E172)

Carnauba wax

6.2 Incompatibilities

Not applicable

6.3 Shelf-life

Three years

6.4 Special precautions for storage

No special precautions for storage

6.5 Nature and content of container

28, 50, and 84, tablets packed in PVC/PVDC blisters or in all aluminium blisters in cardboard cartons.

6.6 Instruction for use and handling, and disposal (if appropriate)

Not applicable

7. MARKETING AUTHORISATION HOLDER

Sanofi Pharma Bristol-Myers Squibb SNC
174 Avenue de France
75013 Paris - France

8. NUMBER IN THE COMMUNITY REGISTER OF MEDICINAL PRODUCTS

EU/1/98/069/001a - Cartons of 28 tablets in PVC/PVDC blisters
EU/1/98/069/001b - Cartons of 28 tablets in all aluminium blisters
EU/1/98/069/002a - Cartons of 50 tablets in PVC/PVDC blisters
EU/1/98/069/002b - Cartons of 50 tablets in all aluminium blisters
EU/1/98/069/003a - Cartons of 84 tablets in PVC/PVDC blisters
EU/1/98/069/003b- Cartons of 84 tablets in all aluminium blisters

9. DATE OF FIRST AUTHORISATION/RENEWAL OF THE AUTHORISATION

15 July 1998

10. DATE OF REVISION OF THE TEXT

LABELLING

Outer packaging text

PLAVIX 75 mg, film-coated tablet

Clopidogrel

Each tablet contains:

Clopidogrel 75 mg present as clopidogrel hydrogen sulphate.

Excipients including lactose, colouring agents (E171, E172)

28 film-coated tablets

Before use, please see package leaflet.

Keep out of the reach of children.

Medicinal product subject to medical prescription.

For oral use.

Expiry date:

Batch number:

EU/1/

Marketing Authorisation Holder:

Sanofi Pharma Bristol-Myers Squibb SNC

174 Avenue de France - 75013 Paris - France

Outer packaging text

PLAVIX 75 mg, film-coated tablet

Clopidogrel

Each tablet contains:

Clopidogrel 75 mg present as clopidogrel hydrogen sulphate.

Excipients including lactose, colouring agents (E171, E172)

50 film-coated tablets

Before use, please see package leaflet.

Keep out of the reach of children.

Medicinal product subject to medical prescription.

For oral use.

Expiry date:

Batch number:

EU/1/

Marketing Authorisation Holder:

Sanofi Pharma Bristol-Myers Squibb SNC

174 Avenue de France - 75013 Paris - France

Outer packaging text

PLAVIX 75 mg, film-coated tablet

Clopidogrel

Each tablet contains:

Clopidogrel 75 mg present as clopidogrel hydrogen sulphate.

Excipients including lactose, colouring agents (E171, E172)

84 film-coated tablets

Before use, please see package leaflet.

Keep out of the reach of children.

Medicinal product subject to medical prescription.

For oral use.

Expiry date:

Batch number:

EU/1/

Marketing Authorisation Holder:

Sanofi Pharma Bristol-Myers Squibb SNC

174 Avenue de France - 75013 Paris - France

Blister text

PLAVIX 75 mg film-coated tablets

Clopidogrel

Sanofi Pharma Bristol-Myers Squibb SNC

Expiry date:

Batch number:

SCIENTIFIC DISCUSSION

Name of the medicinal product:	Plavix 75 mg film coated tablets Sanofi Pharma Bristol-Myers Squibb SNC 174 Avenue de France
Marketing Authorisation Holder:	75013 Paris - France
Active substance:	Clopidogrel hydrogen sulphate Clopidogrel
International Nonproprietary Name:	Platelet aggregation inhibitors excl. Heparin, ATC Code: B01 A C04
Pharmaco-therapeutic group (ATC Code):	
Therapeutic indication(s):	<p>Reduction of atherosclerotic events (myocardial infarction, stroke, death due to vascular causes) in patients with a history of symptomatic atherosclerotic disease defined by ischaemic stroke (from 7 days until less than 6 months), myocardial infarction (from a few days until less than 35 days) or established peripheral arterial disease.</p> <p>This indication is based on the results of the CAPRIE study comparing clopidogrel with acetyl salicylic acid (ASA). The slight but statistically significant difference of clopidogrel over ASA was mainly related to patients enrolled due to peripheral arterial disease. For further information please refer to section 4.4 Special warnings and special precautions for use and 5.1 Pharmacodynamic properties.</p>

1. Introduction

Atherosclerosis is a major pathological process causing death and disability in the Western World. The initial clinical presentation of the atherosclerotic disease depends upon the vascular bed where the atherosclerotic process is the most advanced.

Therapeutic interventions are usually aimed at the vascular territory causing the clinically symptomatic disease.

Patients who suffered from an ischaemic event such as myocardial infarction, stroke or who have peripheral arterial disease with intermittent claudication, represent a group who is at the highest risk of further atherosclerotic/thrombotic events. Prevention of vascular ischaemic events can be achieved with oral anticoagulation or through inhibition of platelet aggregation. Furthermore life style modifications are encouraged (dietary changes, lipid control, exercise and smoking cessation).

Inhibition of platelet aggregation with acetyl salicylic acid (ASA), besides being more practical and safer than anticoagulation, has been shown to be effective. The meta-analysis conducted by the Antiplatelet Trialists' Collaboration (APTC) included 145 randomised trials, involving 100,000 patients at risk of vascular events. Of these, over 70,000 were considered high-risk. The results showed a risk reduction of 25% of vascular events in all high-risk subgroups. The doses of ASA were in the range of 75 to 325 mg daily. There is no evidence that higher doses of ASA confer an increased benefit.

When considering such long-term therapy, the safety and tolerability of the anti-platelet agent is a major consideration. ASA is associated with an increased risk of gastrointestinal ulceration and haemorrhage. Ticlopidine, another commonly used antiplatelet agent, has a higher rate of diarrhoea and rash versus ASA which albeit not clinically serious, can cause drug withdrawal. Although infrequently, ticlopidine causes neutropaenia and thrombocytopenia which can be serious and usually appear in the first three months of long-term therapy.

Plavix tablets contain clopidogrel, a new thienopyridine molecule analogue of ticlopidine, which has been developed as an inhibitor of platelet aggregation for use in the prevention of vascular ischaemic events in patients with established atherosclerotic disease.

2. Part II: Chemical and pharmaceutical aspects

Composition

Plavix is formulated as conventional tablets containing 97.9 mg of clopidogrel hydrogen sulphate equivalent to 75 mg of clopidogrel base, and conventional excipients. The final formulation was optimised from different tablet and one capsule formulations used in early clinical trials.

The medicinal product is supplied in clear or opaque PVC/PVDC blister packs sealed with aluminium foil or all aluminium blisters packs in cardboard cartons of 28, 50, and 84 tablets.

Pharmaceutical development

Clopidogrel is the S-enantiomer of a new thienopyridine molecule analogue containing one single chiral centre. The chemical stability and compatibility of different excipients with four different clopidogrel salts were investigated. The final choice was given to clopidogrel hydrogen sulphate, which has optimal stability and compatibility.

The first pharmaceutical formulation used in the clinical trials was a capsule. In view of marketing, a tablet form was developed and was film-coated to mask the very bitter taste of the drug substance. The tablet formulation intended for marketing was considered to be optimal. Further slight modifications were introduced after the final bioequivalence study, but these did not affect the tablets dissolution characteristics.

Method of preparation

The manufacturing formula for the film-coated tablets was provided. Validation data from 35 semi-industrial batches and 1 industrial batch demonstrated that the manufacturing process is under control and ensures both, batch to batch reproducibility and compliance with specifications.

Control of starting materials

The synthetic pathway is presented as a Drug Master File. The manufacturers of the active substance are Sanofi Chimie and Orgamol LTD companies. The analytical methods used to control starting materials as well as intermediates of synthesis are acceptable and identical for both manufacturers. The impurity profile is well characterised and in line with current ICH guidelines. All other ingredients entering in the preparation of the tablets are adequately controlled.

The synthetic route, control tests and specifications of clopidogrel hydrogen sulphate are acceptable and identical for both manufacturers. The quality of the active substance is guaranteed by the established specifications and the proposed analytical methods are adequately validated.

Control tests on the finished product

The analytical methods are suitable to ensure consistent quality of the finished product. No degradation products have been detected upon storage.

Stability

Drug substance

Results from primary stability studies and additional stability data provided (3 semi and industrial batches, 24 months at 25°C/60% RH and at 30°C/60% RH) support a retest period of 3 years.

Finished product

Results from primary stability studies performed on 3 batches for 36 months at 25°C/60% RH, together with supportive stability studies clearly justifies the 3 years shelf-life. The end of shelf-life specifications were adjusted to tightened values following discussion with the company. The company submitted data available at three years stability studies at 25°C/60% RH.

3. Part III: Pharmacological and toxicological aspects

Pharmacodynamics

The antiaggregating activity was evaluated in *ex vivo* and *in vitro* models. Oral and intravenous administration of clopidogrel inhibited the *ex vivo* ADP (adenosine diphosphate) induced aggregation of platelets, thereby affecting ADP-dependent activation of the GPIIb-IIIa complex, the major receptor for fibrinogen on the platelet surface. This effect was observed in all animal species investigated (mouse, rat, rabbit and baboon). The R-enantiomer was completely inactive and the S-enantiomer was approximately twice more potent than the racemic mixture. No effect on platelet aggregation was observed when clopidogrel was tested *in vitro*. Biotransformation of clopidogrel is necessary to produce inhibition of platelet aggregation.

Clopidogrel exerted an antithrombotic action in various models of thrombosis in rats with a potency at least 50-fold higher than that of ticlopidine. The R-enantiomer of clopidogrel was devoid of any protective action.

The mechanism of the antiaggregating action is related to the specific inhibition of ADP (adenosine diphosphate) receptors on platelets. *In vitro* studies showed that following incubation of clopidogrel with rat liver microsomes, an active metabolite is generated which binds to platelet ADP receptors, thus inhibiting platelet aggregation induced by ADP. This metabolite has been isolated and its structure characterised as a thiol derivative of 2-oxo clopidogrel. The irreversible modification of the ADP-receptor site could be explained by the formation of a disulfur bridge between the reactive thiol and a

cysteine residue of a platelet protein. The company was asked to comment further on the identity of the active metabolite, the site of, and the enzymes involved in, the metabolic activation of clopidogrel. This issue, and its relevance in terms of efficacy and possible interactions was further discussed during the hearing held on 24th February 1998. The CPMP considered that this issue had been satisfactorily addressed by the company and that it was resolved.

Low levels of the R-enantiomer were detected in mice, rats and baboons. This racemisation of S-enantiomer was observed *in vivo*, and comparative toxicology studies revealed that clopidogrel was less toxic than the racemic compound and the R-enantiomer. Metabolic inversion was, however, insignificant in human subjects.

Safety pharmacology studies did not reveal any relevant effects to the central nervous, cardiovascular, respiratory, gastrointestinal and renal systems.

Pharmacokinetics

Metabolism and disposition of clopidogrel were assessed from several *in vitro* and *in vivo* studies.

Studies conducted in the rat with ¹⁴C-labelled clopidogrel showed that 60% of clopidogrel was absorbed in the intestine within 60 minutes. Clopidogrel undergoes extensive first-pass metabolism and the main metabolite found in plasma, the carboxylic acid derivative, is inactive. The peak plasma concentration values were observed 1 to 2 hours after dosing. Administration with food decreased the maximal plasma concentration, but did not affect the AUC.

An extensive binding of clopidogrel and the main circulating metabolite to plasma proteins was observed (87% in mice, 95% in rats and 92% in baboons). Plasma radioactivity was partly covalently bound to plasma proteins, and in the baboon the decrease of this binding was characterised by a half-life of approximately 8 days. Clopidogrel and its carboxylic acid derivative showed little affinity for red blood cells (<20 %).

Clopidogrel was widely distributed in the tissues. In a repeated dose study in rats, accumulation of radioactivity in most of the organs raised over the duration of the study (21 days). Radioactivity was slowly eliminated from the tissues, but 21 days after the suspension of clopidogrel several tissues (arterial wall, thyroid gland, cartilage, skin, spleen) maintained radioactivity levels similar to those observed on the last day of administration.

Clopidogrel undergoes extensive metabolism. Twenty metabolites were identified. The main circulating compound was the carboxylic acid derivative of clopidogrel (SR 26334), which is inactive.

The major route of elimination is biliary. The majority of radioactivity was excreted within 48 hours. A significant entero-hepatic recirculation of radioactivity was observed. It accounted for 45-95% of clopidogrel derived activity and had no effect on platelet aggregation.

In lactating rats, clopidogrel and/or its metabolites levels in milk were 0.5 to 2.6 times higher than the maternal plasma levels. Transfer of the radioactivity to the developing foetus was observed in pregnant rats.

Toxicology

Single dose toxicity of clopidogrel was evaluated in the rat, mouse and in the baboon. Repeated dose toxicity by the oral route were conducted in the mouse for up to 3 months, in the rat for up to 52 weeks and in the baboon for up to one year.

After single oral administration to rats, mice and baboons, toxicity occurred only at very high doses. The target organs were mainly the gastrointestinal tract, the kidney and the lung. After intravenous administration to rats and mice, the main target organs were the kidney and the lung.

The toxicity on the digestive tract was also shown in the repeated dose toxicity studies; in particular in rats and baboons treated orally with high doses (from 400 mg/kg/day and upwards).

The main toxicological finding at doses up to 400 mg/kg/day corresponded to increased liver weight associated with hypertrophy of the smooth endoplasmic reticulum in centrilobular hepatocytes

corresponding to an effect on hepatic enzymes. The no-effect level, based on increased liver weight, were 27 mg/kg/day in rats and 65 mg/kg/day in baboons and correspond to an exposure of at least 7 times (rats) and more than 10 times (baboons) higher than that observed in humans at the recommended therapeutic dose

A slight decrease in heart rate and a slight increase in QT interval were observed in rats and baboons at very high doses (1,000 mg/kg/day in rats, and 400 mg/kg/day in baboons). However, there was no prolongation of QTc-interval. An *in vitro* electrophysiological study on rabbit Purkinje fibres was submitted with the responses to the consolidated list of questions. Although the results did not reveal a proarrhythmic potential, the highest concentration tested (~ 9.6 mg/l) was only three times the C_{max} obtained in humans with the therapeutic dose (~ 2.7 mg/l). This issue and its clinical relevance were further discussed during the hearing held on 24th February 1998. The CPMP considered that this issue had been satisfactorily addressed by the company and that it was resolved.

The R-enantiomer of clopidogrel revealed a higher toxicity than the active compound, namely on the central nervous system. The impact of the toxicity of this impurity on the overall toxicological profile of clopidogrel formulation seems to be minor due to its low concentration in the test compound. Furthermore, there is no evidence of epimerisation in man.

The reproduction toxicity studies in rats and rabbits did not reveal any teratogenic or foetotoxic potential for clopidogrel even at borderline maternally toxic doses. Male and female fertility, growth and development of the F1 offspring were not affected. High doses of clopidogrel induced a slight delay in the development of the offspring of lactating rats possibly due to the effect of the drug excreted in the lactating milk. The effect was reversible after weaning. In the light of these findings, and in the absence of significant experience in pregnant women, clopidogrel is contraindicated during breast-feeding and not recommended during pregnancy.

A battery of *in vitro* studies and one *in vivo* mouse micronucleus assay did not reveal any mutagenic, genotoxic or clastogenic potential of clopidogrel. No immunogenic, antigenic, phototoxic or photoallergenic potential was observed.

The carcinogenic potential of clopidogrel was investigated in two life-span studies in the rat and in the mouse. Both studies were negative. The company was requested to discuss the clinical relevance of the increased incidence of thyroid cysts observed in the rat carcinogenicity study taking into account the high level of sustained radioactivity observed in the thyroid gland in the tissue distribution studies. The company stated that developmental cysts are embryonic vestiges and do not represent lesions with neoplastic potential. This explanation was considered satisfactory.

4. Part IV: Clinical aspects

The clinical documentation includes an extensive clinical programme of 51 clinical pharmacology studies involving 1,150 subjects and one comparative international multicentre clinical trial (the "CAPRIE" study, Clopidogrel versus ASA in Patients at Risk of Ischaemic Events) involving 19,185 patients.

Human pharmacology

The pharmacodynamic effects of clopidogrel in humans were evaluated in several studies both in healthy subjects and in patients, after single or repeated administration. The selection of the dose was based on two surrogate markers of pharmacological activity, inhibition of ADP-induced platelet aggregation and prolongation of bleeding time.

The precise correlation between the degree of inhibition of ADP-induced platelet aggregation and the reduction in ischaemic events is unknown. Dose selection for clopidogrel therefore aimed to identify that dose which inhibits ADP-induced platelet aggregation and prolongs bleeding time to the same extent as the proven effective dose of ticlopidine (250 mg twice daily).

Results from three phase I dose ranging studies indicated that a daily dose of 75 mg of clopidogrel would give the same level of ADP-induced platelet aggregation and prolongation of bleeding time as 250 mg of ticlopidine twice daily.

These findings were confirmed in a study performed in 150 atherosclerotic patients. The pharmacological activity of repeated administration (28 days) of 5 dose levels (10-100 mg per day) was compared to ticlopidine (250 mg twice a day) and placebo. The dose of 75 mg once daily was superior to 50 mg, and comparable to 100 mg once daily in terms of inhibition of ADP-induced platelet aggregation.

Based on the results of the above studies, it was considered that a daily dose of 75 mg clopidogrel would be effective and well tolerated in the target population. Consequently this dose was chosen for the phase III clinical programme.

Clopidogrel selectively inhibits the binding of ADP to its platelet receptor, and the subsequent ADP-mediated activation of the GPIIb/IIIa complex, thereby inhibiting platelet aggregation. Dose dependent inhibition of platelet aggregation is observed 2 hours after single oral dose of clopidogrel. Repeated doses of 75 mg per day inhibit ADP-induced platelet aggregation on the first day, and inhibition reaches steady state between day 3 and day 7. At steady state, the average inhibition level was between 40% and 60%.

The pharmacological activity is maintained with long-term use of clopidogrel. One study showed that the level of inhibition of platelet aggregation and prolongation of bleeding time were similar at one week and after 80 days of treatment. After discontinuation, inhibition of platelet aggregation and bleeding time returned to baseline values approximately 5 days after the last dose.

These findings are consistent with the mode of action of clopidogrel, namely that the active metabolite binds irreversibly to the platelet ADP receptor, therefore affecting the platelets for the remainder of their life span.

Pharmacokinetics

The pharmacokinetic profile was evaluated in 27 clinical trials carried out in healthy volunteers and special populations with either single or repeated administration.

Clopidogrel is rapidly absorbed and extensively metabolised by the liver. However, plasma levels of the parent compound are very low and below the quantification limit (0.00025 mg/l) beyond 2 hours. The main circulating metabolite, which is inactive, is the carboxylic acid derivative which represents about 85% of the circulating compound in plasma. Peak plasma levels of this metabolite (approx. 3 mg/l after repeated 75 mg oral doses) occurred approximately 1 hour after dosing.

Clopidogrel is a prodrug. The active metabolite, a thiol derivative, is formed by oxidation of clopidogrel to 2-oxo-clopidogrel and subsequent hydrolysis. The oxidative step is regulated primarily by Cytochrome P₄₅₀ isoenzymes 2B6 and 3A4 and to a lesser extent by 1A1, 1A2 and 2C19. The active thiol metabolite, which has been isolated *in vitro*, binds rapidly and irreversibly to platelet receptors, thus inhibiting platelet aggregation. This metabolite has not been detected in plasma.

Consequently, though useful as a marker of absorption, the relevance of conventional pharmacokinetic studies of this inactive metabolite is limited. Thus, many aspects of the behaviour of clopidogrel, in special populations or potential interactions with other drugs, were studied using the dynamic markers, inhibition of ADP-induced platelet aggregation and prolongation of bleeding time.

In vitro studies showed that clopidogrel and SR 26334 bind reversibly to plasma proteins (98 % and 94 % respectively). Due to this high level of binding and the potential for interactions with products with a known affinity for albumin, specific *in vitro* interaction studies were performed. These studies showed that there was no potential for interaction with such products.

A low level of covalent binding to proteins was observed in *in vivo* studies. Theoretically covalent binding to proteins could result in allergic reactions, but specific immunotoxicity studies in animals did not indicate clopidogrel had the potential to cause such effects.

The elimination half-life of SR 26334 after a single and repeated administration of 75 mg once daily of clopidogrel is approximately 8 hours.

The excretion of clopidogrel following a single dose of 75 mg of ¹⁴C clopidogrel given alone or at the end of a ten-day dosing period of unlabelled drug accounted for 92-98% of the radioactive dose administered, equivalent percentages excreted through the faecal and urinary routes (46 and 50% respectively). The terminal half-life of radioactivity was approximately 7 days. This relatively long half-life is consistent with the platelet turnover.

Interactions

The interaction between clopidogrel and several other drugs (e.g. heparin, non-steroidal anti-inflammatory drugs, theophylline and digoxine) has been well investigated. The findings are reflected in the summary of product characteristics and in the package leaflet.

Hepatic microsomal studies indicated that clopidogrel could inhibit one of the cytochrome P₄₅₀ enzymes, CYP 2C9. This could lead to elevated plasma levels of drugs, which are substrates for this isoenzyme (e.g. phenytoin, warfarin, tolbutamide and certain non-steroidal anti-inflammatory drugs such as piroxicam and diclofenac). Furthermore *in vitro* studies on human liver microsomes showed that the main circulating compound of clopidogrel could minimally inhibit one of the two main pathways through which glibenclamide is cleared. Data from the CAPRIE study indicate that phenytoin, tolbutamide and glibenclamide can be safely coadministered with clopidogrel.

Two studies were undertaken to assess the potential interaction of ASA with clopidogrel. ASA did not modify the clopidogrel-mediated inhibition of ADP-induced platelet aggregation, but clopidogrel potentiated the effect of ASA on collagen-induced platelet aggregation. However, concomitant administration of 500 mg of ASA twice a day for one day did not significantly increase the prolongation of bleeding time induced by clopidogrel intake. The safety of the chronic concomitant administration of ASA and clopidogrel has not been established.

Due to the use of too high a dose of warfarin (30-40 mg), the study to assess the potential interaction of warfarin with clopidogrel was prematurely discontinued. Although this study was inconclusive it is reasonable to assume that the risk of bleeding would be increased by the association of these two agents. Consequently, the concomitant administration of clopidogrel with warfarin can not be recommended.

The experience with the coadministration of clopidogrel with thrombolytic agents is limited. In one open uncontrolled study regrouping 116 patients, the concomitant administration of clopidogrel, rt-PA and heparin was assessed in patients with recent myocardial infarction. The incidence of moderate to severe bleeding was 1.7%. No data exist for the concomitant administration of clopidogrel with other thrombolytic agents. One interaction study of combined administration of heparin/clopidogrel and heparin/ASA resulted in one case of bleeding and 4 cases of haematoma at the injection site for the heparin/clopidogrel group (n=15) compared to no bleeding events in the heparin/ASA group (n=13). However, it should be noted that clopidogrel treatment started with a loading dose of 375 mg on day 1 followed by a dose of 75 mg daily.

Special populations

A small comparative study without control groups showed that there is no basis for expecting any differences in terms of efficacy and safety in patients with renal impairment. No dosage adjustment of clopidogrel is required. The limited relevant clinical experience regarding the therapeutic use of clopidogrel in renally impaired patients was further addressed during the hearing on 24th February 1998. However in view of the limited data and the fact that patients with severe renal impairment were excluded from the CAPRIE study, clopidogrel should be used with caution in patients with renal impairment.

The company provided results of a pharmacokinetics/pharmacodynamics study of clopidogrel in 12 subjects with cirrhosis, compared with 12 healthy volunteers. As expected from the extensive first pass metabolism, the plasma level of clopidogrel was markedly elevated in cirrhotic patients. The inhibitory effect on platelets and the incidence of adverse events were globally comparable between the two groups. The mean inhibition of platelet aggregation was similar with a much larger variability in patients with hepatic impairment. The limited relevant clinical experience regarding the therapeutic use of clopidogrel in hepatically impaired patients was further addressed during the hearing on 24th February 1998.

Taking into consideration that patients with hepatic impairment were excluded from the CAPRIE study, that they may have bleeding diatheses, and that liver metabolism plays a key role in the generation of the active metabolite, clopidogrel is contraindicated in patients with severe liver impairment. Furthermore, clopidogrel should be used with caution in patients with moderate liver impairment who may have bleeding diatheses.

Clinical experience

Design

CAPRIE was a randomised, international multicentre double blind comparative trial designed to assess the relative efficacy of clopidogrel (75 mg once daily) and ASA (325 mg once daily) in reducing the risk of a composite outcome cluster of ischaemic stroke, myocardial infarction, or vascular death. The relative safety was also assessed.

The **primary efficacy endpoint** was based on the first occurrence of an event in the composite outcome cluster of fatal or non-fatal ischaemic stroke, fatal or non-fatal myocardial infarction and vascular death.

The CAPRIE study included 19,185 patients with atherothrombosis as manifested by recent ischaemic stroke (IS) (between 7 days and 6 months), recent myocardial infarction (MI) (<35 days), or established peripheral arterial disease (PAD).

9,599 (50.0%) were assigned to receive clopidogrel and 9,586 (50.0%) were assigned to receive ASA. The two groups were similar with respect to demographic characteristics, although there was a slight but statistically significant imbalance in the proportion of non-Caucasians in the two groups ($p=0.02$). The mean age was 62.5 (standard deviation 11.08) years with a range from 21 to 94 years.

The median duration of participation was between 20.4–23.9 months. 2,286 patients (23.8%) who received clopidogrel and 2,311 patients (24.1%) who received ASA permanently discontinued the study drug early. The majority of cases were due to adverse events or to patients who withdrew their consent for study participation.

Results and discussion

Clopidogrel significantly reduced the incidence of new ischaemic events (combined end point of myocardial infarction, ischaemic stroke and vascular death) compared to ASA. In the intention to treat analysis, 939 events were observed in the clopidogrel group and 1,020 events with ASA (relative risk reduction (RRR) 8.7%, [95% CI: 0.2 to 16.4]; $p=0.045$). This corresponds for every 1,000 patients treated for 2 years, to 10 [CI: 0 to 20] additional patients being prevented from experiencing a new ischaemic event. The cumulative proportion of patients who experienced an event in the primary outcome cluster shows that the benefit of clopidogrel over ASA appears at an early stage of the treatment and continues to increase over time.

Two secondary endpoint clusters (ischaemic stroke, myocardial infarction, amputation, or vascular death; and vascular death alone) showed that there were more events in the ASA group than in the clopidogrel group, although the differences were not statistically significant. Analysis of total mortality as a secondary endpoint did not show a significant difference between clopidogrel (5.8%) and ASA (6.0%).

The CAPRIE trial was powered to detect a realistic treatment effect in the whole study cohort but not in each of the three clinical subgroups.

A test for heterogeneity of the three treatment effects was statistically significant suggesting that the true benefit may not be identical across the three clinical subgroups. In a subgroup analysis by qualifying condition (myocardial infarction, ischaemic stroke, and PAD), the benefit appeared to be strongest (achieving statistical significance at $p=0.003$) in patients enrolled due to PAD (especially those who also had a history of myocardial infarction) (RRR= 23.7%; CI : 8.9 to 36.2) and weaker (not significantly different from ASA) in stroke patients (RRR= 7.3% ; CI : -5.7 to 18.7). In patients who were enrolled in

the trial on the sole basis of a recent myocardial infarction, clopidogrel was numerically inferior, but not statistically different from ASA (RRR= -4.0%; CI: -22.5 to 11.7).

Since the CAPRIE trial was not powered to evaluate efficacy of individual subgroups, it is not clear whether the differences in relative risk reduction across qualifying conditions are real, or a result of chance.

This issue of subgroup heterogeneity regarding qualifying conditions, and the implications for the therapeutic indication with regard to efficacy and especially safety (all cause mortality, vascular death, sudden death) in the myocardial infarction subgroup were further discussed during the hearing held on 24th February 1998, and during the March CPMP meeting. The final recommendation regarding initiation of clopidogrel in patients with myocardial infarction agreed by the CPMP is reflected in the agreed therapeutic indication.

Using the appropriate trials from the APTC meta-analysis (i.e. those in the same clinical syndromes), the efficacy of clopidogrel versus a putative placebo was estimated. These analyses strongly supported the superiority of clopidogrel over placebo in the overall CAPRIE population and in the IS and MI subgroup.

Oral explanations were provided during the hearing held on 24th February 1998 on the time to onset of action in view of the lack of new data to document the efficacy of clopidogrel at the first few days of an acute situation. The final recommendation agreed by the CPMP is that clopidogrel should not be initiated within the first few days following myocardial infarction, and that in view of the lack of data, clopidogrel can not be recommended in unstable angina, PTCA (stenting), CABG and acute ischaemic stroke (less than 7 days).

An unplanned subgroup-analysis by age suggested that the benefit of clopidogrel in patients over 75 years was less than that observed in patients ≤ 75 years. There were 243 patients with events in the clopidogrel group compared to 220 in the ASA group (efficacy odds ratio: 1.176; CI: 0.980 to 1.412). Efficacy and safety in patients over 75 years of age, together with the possible interaction with the qualifying condition of myocardial infarction, and effects on sudden deaths were addressed during the hearing held on 24th February 1998. The issue was considered as satisfactorily addressed by the CPMP and no specific recommendation was introduced in the Summary of Product Characteristics for patients over 75 years of age.

Safety

Clinical pharmacology studies

The safety profile of clopidogrel was based on the experience from the CAPRIE study and also from 51 clinical pharmacology studies including 1,150 healthy volunteers and patients receiving clopidogrel, placebo and/or another drug.

As expected from its pharmacological action, platelet clotting and bleeding disorders were more common on clopidogrel (10% at 75 mg once daily compared to 3.9% on placebo). Haematoma and purpura, including bruising were the other most common events. Gastrointestinal tract adverse events occurred in 10.5% compared to 7.1% on placebo, though diarrhoea was the only individual event seen more frequently on clopidogrel (2.8% at 75 mg once daily compared to 0.7% on placebo). Its incidence did not increase with higher doses and was rarely severe. Rash was noted in 1% of subjects on 75 mg once daily and 2.5% at higher doses compared to 0.7% on placebo.

The clinical pharmacology studies showed that adverse events commonly seen with ticlopidine such as diarrhoea and rash were less common with clopidogrel. Severe cases were rare. More importantly there was no evidence of clinically relevant thrombocytopaenia, neutropaenia or impairment of liver function.

CAPRIE study

The extent of exposure to clopidogrel 75 mg once daily as well as to ASA 325 mg once daily in the CAPRIE study provided a strong basis for a reliable comparison of the safety profile of the two drugs.

The mean treatment duration was 19.5 months (Standard deviation 10.18 months for both treatment groups) allowing over 15,500 patient years experience with each drug. Only 56 patients were lost to follow-up: 30 on clopidogrel and 26 on ASA.

A comparable rate of adverse events was reported under the two treatments: 86.3% on the clopidogrel group and 86.5% patients on the ASA group reported at least one adverse event. This was expected given the length of the treatment and the severity of the underlying condition.

The overall frequency of serious adverse events was similar for both treatment groups (40.4% on clopidogrel and 41.1% on ASA). The incidence of death resulting from an adverse event that began under treatment was similar in both treatment groups: 4.1% and 4.3% on clopidogrel and ASA respectively. Very few deaths were considered as related to the study drug : 11 on clopidogrel and 13 on ASA.

Gastrointestinal adverse events were statistically more frequent in the ASA group ($p<0.001$). Diarrhoea was rarely severe and was reported at a higher frequency on clopidogrel compared to ASA (4.5% vs. 3.4%).

Cardiovascular system ($p=0.002$), central and peripheral nervous ($p=0.016$), heart rate and rhythm ($p=0.011$) and red blood cell ($p=0.024$) disorders were significantly more frequent in the ASA group than in the clopidogrel group .

There was no statistically significant difference between treatments groups in the incidence of adverse events in the urinary, biliary and hepatic systems.

There were no differences between treatments groups in the frequency of potentially clinically significant liver function abnormalities (AST, ALT and alkaline phosphatase), although the clopidogrel group had a small mean increase in total bilirubin (0.01-0.03 mg/dl) relative to ASA.

Skin and appendage disorders were more frequent in the clopidogrel group ($p<0.001$) than in the ASA group. Specifically, rash was experienced by significantly more patients in the clopidogrel group than in the ASA group ($p=0.012$). Patients in the clopidogrel group experienced significantly more pruritus ($p<0.001$).

Hyperuricaemia ($p=0.015$) was experienced by significantly more patients in the ASA group. Asthenia ($p=0.017$) and gout ($p=0.026$) were experienced by significantly more patients in the clopidogrel group.

The percentage of patients with a platelet, bleeding or clotting disorder did not differ significantly between treatment groups. However significantly more patients experienced purpura (bruising) in the clopidogrel group (5.27%) than in the ASA group (3.68%) ($p<0.001$).

The overall incidence of neutropaenia were low and did not differ between groups. Neutropaenia was seen in 0.08% of the clopidogrel group and in 0.15% of the ASA group. Only 4 cases (0.04%) on clopidogrel and 2 cases (0.02%) on ASA had severe low neutrophil count below $0.450 \times 10^9/l$. Two cases of agranulocytosis occurred in patients taking clopidogrel.

No significant difference between clopidogrel and ASA was found either in the frequency of thrombocytopenia (<100 G/L) 0.33% in both groups, or the frequency of severe (<80 G/L) thrombocytopenia (0.16% vs. 0.08%).

One serious unexpected case of aplastic anaemia occurred during the CAPRIE study and seemed probably related to clopidogrel. The patient however was also on other medication known to be associated with haemotoxic effects. The patient was not rechallenged with clopidogrel.

The overall incidence of any bleeding did not differ statistically significantly between the two groups (9.3 % in both groups). The occurrence of gastrointestinal bleeding was slightly but significantly higher in the ASA group (2.7% versus 2%, $p=0.002$). Three patients died from gastrointestinal bleeding (one in the clopidogrel group and two in the ASA group). There was no statistically significant difference in the incidence of intracranial bleeding.

The incidence of other bleeding was higher in patients that received clopidogrel compared to ASA (7.3% vs. 6.5%). However, the incidence of severe events was similar in both treatment groups

(0.6% vs. 0.4%). The most frequently reported events in both treatment groups were: purpura/bruising/haematoma, and epistaxis. Other less frequently reported events were haematoma, haematuria, and eye bleeding (mainly conjunctival).

Of the 75 patients in the clopidogrel group with eye bleeding only five had a severe event. In the ASA group, 46 patients experienced eye bleeding with only one patient having a severe event.

A substantial number of patients (clopidogrel n=382, ASA n=387) underwent cardiac catheterisation with angiography, PTCA or stenting. A slight excess of bleeding events (clopidogrel n=46, ASA n=38) or haematoma (clopidogrel n=18, ASA n=14) was seen in the clopidogrel treatment group as compared to the ASA group. Another clinical study has evaluated the safety of clopidogrel compared to aspirin in patients undergoing PTCA. In this study a higher incidence of bleeding or haematoma was observed in the clopidogrel group (5/15 patients versus 0/13). It should however be noted that clopidogrel was given as loading dose of 375 mg followed by 75 mg once daily.

The post-marketing experience confirms the safety profile defined during the clinical development; hypersensitivity reactions have been reported: these mainly include skin reactions (maculopapular or erythematous rash, urticaria....) and/or pruritus. Very rare cases of bronchospasm, angioedema or anaphylactoid reactions have been observed.

5. Overall conclusion and benefit/risk assessment

Benefit risk assessment

The CAPRIE trial showed that clopidogrel at a dose of 75 mg once daily is an effective antithrombotic agent which reduced by 8.7% ($p=0.045$) compared to ASA (325 mg once daily), the incidence of new ischaemic events (combined end point of myocardial infarction, ischaemic stroke and vascular death) in patients with clinical evidence of atherosclerosis. In the intention to treat analysis, 939 events were observed in the clopidogrel group and 1,020 events with ASA (relative risk reduction (RRR) 8.7%, [95% CI: 0.2 to 16.4]; $p=0.045$), which corresponds for every 1,000 patients treated for 2 years, to 10 [CI: 0 to 20] additional patients being prevented from experiencing a new ischaemic event.

The safety profile shows that clopidogrel is at least as well tolerated as ASA. Overall clopidogrel was well tolerated, having an adverse event profile comparable to ASA, but with better gastrointestinal tolerability. Only rash, purpura (bruising) and diarrhoea were notable in the clopidogrel group but were rarely severe. There is no evidence that clopidogrel shares the risk, seen with ticlopidine, of neutropaenia or thrombocytopaenia. The company has additionally been requested to provide as a follow-up to the marketing authorisation together with the periodic safety update reports an analysis of the haematological effects of clopidogrel.

Conclusion

The quality of the medicinal product is considered satisfactory. No major objections on the chemical and pharmaceutical aspects of the dossier prevent the approval of the medicinal product. However a number of follow-up measures have been addressed by the applicant.

The pharmacodynamic activity of clopidogrel was adequately evaluated. Clopidogrel is a prodrug. The active metabolite binds rapidly and irreversibly to platelet receptors, thus inhibiting platelet aggregation.

The overall animal toxicological profile of clopidogrel (hydrogen sulphate salt form) was adequately evaluated and no major findings were described at doses at least up to 100 mg/kg/day in all species. The systemic exposure of the several animal species to that dose as compared to the human exposure expected at therapeutic dose of 75 mg/day is satisfactorily higher and does not suggest safety concerns in relation to the human use of the drug.

Although the explanation provided by the company regarding the increased incidence of thyroid cysts in rats may be acceptable, no further explanation was provided on the high level of sustained radioactivity observed in the tissue distribution studies. Therefore, the company is requested to provide as a post marketing surveillance follow-up measure clinical safety data on the thyroid function.

Clinical efficacy is based on the results of the CAPRIE trial which showed that clopidogrel at a dose of 75 mg once daily reduces by 8.7% ($p=0.045$) the incidence of new ischaemic events (combined end point of myocardial infarction, ischaemic stroke and vascular death) in patients with clinical evidence of atherosclerosis, over ASA (325 mg once daily). Overall clopidogrel was well tolerated, having an adverse event profile comparable to ASA, but with better gastrointestinal tolerability.

The CPMP considered the benefit/risk ratio to be favourable and issued on 25 March 1998 a positive opinion for granting a marketing authorisation for Plavix.

I BACKGROUND INFORMATION ON THE PROCEDURE

1. Submission of the dossier

The company Sanofi Pharma Bristol-Myers Squibb SNC, France submitted on 9 April 1997 to the European Agency for the Evaluation of Medicinal Products (EMEA) an application for the marketing authorisation of the medicinal product Plavix falling within the scope of Part B of the Annex of the Council Regulation (EEC) 2309/93, of 22 July 1993.

The Rapporteur and Co-rapporteur appointed by the CPMP and the evaluation teams were as follows:

Rapporteur:	Prof. M. Forte	Co-Rapporteur:	Pharm. G. De Greef
Evaluators:	Prof. J. Guimarães Morais	Evaluators:	Dr P. Celis
	Prof. M. Beatriz Lima		Prof. J. M. Boeynaems
	Prof. E. Mota		Prof. J. P. Wautrecht

Licensing status:

Plavix has been licensed in the USA since 17 November 1997.

2. Steps taken for the assessment of the product

- The Rapporteur's assessment report was circulated to all CPMP Members on 28 July 1997. The Co-Rapporteur's assessment report was circulated to all CPMP Members on 25 July 1997.
- The CPMP Consolidated list of questions was adopted on 24 September 1997.
- The responses to the consolidated list of questions were received on 8 December 1997.
- The Joint Rapporteur/Co-Rapporteur assessment report on the responses to the consolidated list of questions was circulated on 21 January 1998.
- The company submitted written responses on the outstanding chemical and pharmaceutical issues on 20 February 1998.
- During its meeting on 24 February 1998, the CPMP agreed on a list of outstanding clinical issues to be addressed by the company in an oral explanation.
- A hearing was held on 24 February 1998, to address the outstanding clinical issues.
- The CPMP, during its meeting on 23-25 February 1998, considered the responses provided by the company, and discussed the recommendations presented by the Rapporteur.
- The CPMP, during its meeting on 23-25 February 1998, considered the responses provided by the company to some of the clinical issues not to be satisfactory. Therefore, the CPMP requested additional written information to be submitted.
- On the basis of the responses provided by the company, the CPMP discussed and amended the Summary of Product Characteristics following additional oral explanations provided by the company on 24 March 1998.
- A letter of undertaking on the follow-up measures as requested by the CPMP, was signed by the applicant on 25 March 1998.
- During the meeting on 25 March 1998, the CPMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation.
- On 15 July 1998, the European Commission issued a Marketing Authorisation for Plavix.

II GENERAL CONDITIONS FOR THE MARKETING AUTHORISATION

1. Manufacturing authorisations

Manufacturers responsible for batch release in the European Economic Area:

Sanofi Winthrop Industrie
1, rue de la Vierge
33340 Ambarès
France

Manufacturing authorisation issued on 19 November 1996 by Agence du Médicament, France.

and

Sanofi Winthrop Ltd, Production Division
Edgefield Avenue, Fawdon
Newcastle Upon Tyne NE3 3TT
United Kingdom

Manufacturing authorisation issued on 14 November 1996 by Medicines Control Agency (Market Towers, 1 Nine Elms Lane, London, SW8 5NQ, United Kingdom)

2. Conditions or restrictions of supply and use

Medicinal product subject to medical prescription.

Steps taken after granting the Marketing Authorisation

- The Marketing Authorisation Holder submitted to the EMEA on 06 November 1998 an application for one type I variation falling within the scope of item No 3 of Annex I to Commission Regulation (EC) No 542/95, as amended. The Marketing Authorisation Holder applied for:
A change in the address of the marketing authorisation holder.
On 03 December 1998, the EMEA approved the variation. The variation required amendments in the relevant sections (annexes I, IIIA and IIIB) of the Commission decision. The European Commission amended the Decision on 01 February 1999.
- The Marketing Authorisation Holder submitted to the EMEA on 29 December 1998 an application for one type I variation falling within the scope of item No 8 of Annex I to Commission Regulation (EC) No 542/95, as amended. The Marketing Authorisation Holder applied for:
A change in the qualitative composition of immediate packaging material.
On 05 February 1999, the EMEA approved the variation. This variation required amendments to annexes I and IIIB of the Commission Decision. Revised EMEA notification dated 01 April 1999 regarding EU numbers. The European Commission amended the Decision on 11 June 1999.
- The Marketing Authorisation Holder submitted to the EMEA on 04 February 1999 an application for one type I variation falling within the scope of item No 1 of Annex I to Commission Regulation (EC) No 542/95, as amended. The Marketing Authorisation Holder applied for:
An alternative manufacturing site for the finished product.
On 22 March 1999, the EMEA approved the variation. This variation required amendments to annexes II and IIIB of the Commission Decision. The European Commission amended the Decision on 16 July 1999.
- The Marketing Authorisation Holder submitted to the EMEA on 04 February 1999 an application for one type I variation falling within the scope of item No 16 of Annex I to Commission Regulation (EC) No 542/95, as amended. The Marketing Authorisation Holder applied for:
A change in the batch size of the finished product.
On 22 March 1999, the EMEA approved the variation. This variation did not require any amendment to the Commission Decision.
- The Marketing Authorisation Holder submitted to the EMEA on 08 April 1999 an application for one type I variation falling within the scope of item No 32 of Annex I to Commission Regulation (EC) No 542/95, as amended. The Marketing Authorisation Holder applied for:
A new engraving on the tablets.
On 13 May 1999, the EMEA approved the variation. This variation required amendments to annexes I and IIIB of the Commission Decision. The European Commission amended the Decision on 08 July 1999.
- The Marketing Authorisation Holder submitted to the EMEA on 08 April 1999 an application for one type I variation falling within the scope of item No 14 of Annex I to Commission Regulation (EC) No 542/95, as amended. The Marketing Authorisation Holder applied for:
A change in specifications of the active substance.
On 06 July 1999, the EMEA approved the variation. This variation did not require any amendment to the Commission Decision.

- On 26 April 1999, the Marketing Authorisation Holder submitted a Type II variation application in accordance with Article 6 of Commission Regulation (EC) No. 542/95 of 10 March 1995, as amended. The scope of the variation related to the update of the Summaries of Product Characteristics and Package Leaflets according to the assessment of the second PSUR

On 20 May 1999 the CPMP approved the variation. The variation required amendments in annexes I and IIIB of the Commission Decision. The European Commission amended the Decision on 07 September 1999.

- Under finalisation.

Exhibit 17

A CLINICAL TRIAL COMPARING THREE ANTITHROMBOTIC-DRUG REGIMENS AFTER CORONARY-ARTERY STENTING

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FOR THE STENT ANTICOAGULATION RESTENOSIS STUDY INVESTIGATORS*

ABSTRACT

Background Antithrombotic drugs are used after coronary-artery stenting to prevent stent thrombosis. We compared the efficacy and safety of three antithrombotic-drug regimens — aspirin alone, aspirin and warfarin, and aspirin and ticlopidine — after coronary stenting.

Methods Of 1965 patients who underwent coronary stenting at 50 centers, 1653 (84.1 percent) met angiographic criteria for successful placement of the stent and were randomly assigned to one of three regimens: aspirin alone (557 patients), aspirin and warfarin (550 patients), or aspirin and ticlopidine (546 patients). All clinical events reflecting stent thrombosis were included in the prespecified primary end point: death, revascularization of the target lesion, angiographically evident thrombosis, or myocardial infarction within 30 days.

Results The primary end point was observed in 38 patients: 20 (3.6 percent) assigned to receive aspirin alone, 15 (2.7 percent) assigned to receive aspirin and warfarin, and 3 (0.5 percent) assigned to receive aspirin and ticlopidine ($P=0.001$ for the comparison of all three groups). Hemorrhagic complications occurred in 10 patients (1.8 percent) who received aspirin alone, 34 (6.2 percent) who received aspirin and warfarin, and 30 (5.5 percent) who received aspirin and ticlopidine ($P<0.001$ for the comparison of all three groups); the incidence of vascular surgical complications was 0.4 percent (2 patients), 2.0 percent (11 patients), and 2.0 percent (11 patients), respectively ($P=0.02$). There were no significant differences in the incidence of neutropenia or thrombocytopenia (overall incidence, 0.3 percent) among the three treatment groups.

Conclusions As compared with aspirin alone and a combination of aspirin and warfarin, treatment with aspirin and ticlopidine resulted in a lower rate of stent thrombosis, although there were more hemorrhagic complications than with aspirin alone. After coronary stenting, aspirin and ticlopidine should be considered for the prevention of the serious complication of stent thrombosis. (N Engl J Med 1998;339:1665-71.)

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THE implantation of coronary stents has become a major form of revascularization therapy for coronary artery disease. In early clinical trials,¹ there were high rates of stent thrombosis (approaching 20 percent), leading to the adoption of an antiplatelet and anticoagulant regimen that included intravenous low-molecular-weight dextran, oral aspirin and dipyridamole, and intravenous

heparin followed by oral warfarin. The incorporation of this aggressive antithrombotic treatment strategy in subsequent randomized clinical trials²⁻⁴ reduced the risk of acute and subacute stent thrombosis to approximately 3.5 percent. However, as compared with conventional balloon angioplasty, stenting with aggressive antithrombotic-drug therapy doubled the length of hospitalization (from three to six days) and increased the rate of hemorrhagic and vascular complications from 3 to 4 percent to 7 to 13 percent.^{2,3,5}

More recently, registry data have demonstrated that the risk of stent thrombosis can be further reduced by the use of a combination of high-pressure, balloon-expandable stents and antithrombotic therapy with aspirin and ticlopidine.⁶⁻⁸ A single-center, randomized trial also demonstrated the superiority of aspirin and ticlopidine over aspirin and warfarin for the prevention of stent thrombosis in high-risk patients.⁹ Moreover, a single-center registry and one small, randomized trial suggested that aspirin alone might be adequate for the prevention of stent thrombosis.^{10,11} There has also been concern about the possibility of neutropenia and thrombocytopenia in association with ticlopidine therapy.¹² We compared the 30-day clinical outcomes for three antithrombotic-drug regimens — aspirin alone, aspirin and warfarin, and aspirin and ticlopidine — after elective coronary-artery stenting.

METHODS

Objectives and Design of the Study and Selection of Patients

The primary objective was to compare the incidence of stent thrombosis in patients with single-vessel or multivessel disease of native coronary arteries who were successfully treated with a high-pressure, balloon-expandable stent at 1 of 50 centers in the United States and who were then randomly assigned to receive one of three antithrombotic-drug regimens. The implantation of a Palmaz-Schatz stent (Cordis, Warren, N.J.) was considered to

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*Other members of the Stent Anticoagulation Restenosis Study are listed in the Appendix.

be successful if the final degree of stenosis within the stent was less than 10 percent (by visual estimate), there was no evidence of thrombus or of dissections (more than grade B according to the National Heart, Lung, and Blood Institute criteria), there was grade 3 flow according to the criteria of the Thrombolysis in Myocardial Infarction study, and no more than two stents were needed to treat one long (≤ 25 mm) lesion or two focal (≤ 12 mm) lesions in one or two native coronary arteries. Patients who did not meet the criteria for successful stenting were enrolled in a prospective trial that was identical to the randomized trial in terms of data collection and follow-up except that patients were not assigned to a specific drug-treatment strategy.

Randomization was not blinded, but all end points were adjudicated by a clinical events committee whose members were unaware of the patients' treatment assignments. The study complied with the Declaration of Helsinki regarding investigations in humans and was approved for an investigational-device exemption by the Food and Drug Administration (FDA), and all investigational sites received approval from their local hospital investigational review boards. All patients gave written informed consent.

Patients were eligible for enrollment if they had one or two target lesions with more than 60 percent stenosis in a 3-to-4-mm native coronary artery, not involving the left main coronary artery or a major coronary bifurcation. Other exclusion criteria were the presence of additional stenoses within the target vessel; recent (within 7 days before enrollment) acute myocardial infarction; known contraindications to the use of aspirin, ticlopidine, or warfarin; a history of bleeding diathesis; current treatment with abciximab; and planned angioplasty of another lesion within 30 days after enrollment.

Eligible patients were randomly assigned in equal proportions with use of a prespecified randomization sequence to one of the three antithrombotic-drug regimens, according to clinical site and history of diabetes mellitus.

Coronary-Stent Procedure

All patients received nongeneric, non-enteric-coated aspirin (325 mg) and intravenous heparin (10,000 to 15,000 U) to maintain an activated clotting time of 250 to 300 seconds during the implantation of the stents. Before the stents were implanted, lesions were treated with balloon angioplasty, directional atherectomy, or rotational atherectomy. The stent was implanted with a stent delivery system (Johnson and Johnson, Warren, N.J.) approved by the FDA that consisted of a specially designed balloon catheter onto which a standard 15-mm Palmaz-Schatz coronary stent had been crimped within a protective nylon sheath. The sizing of the stents followed standard FDA guidelines: the ratio of the diameter of the balloon to the diameter of the artery was 1.1 to 1.0, with a deployment pressure of 6 to 8 atmospheres. The technique used in this study was designed to achieve a residual stenosis of less than 10 percent by visual estimate, which usually required further dilation of the balloon at high pressure (≥ 16 atmospheres) with a separate high-pressure balloon measuring 15 to 20 mm. To reduce the number of variables that might influence stent thrombosis, all procedures were performed with use of the same low osmolar ionic angiographic contrast medium (ioxaglate meglumine, Mallinckrodt, St. Louis).

Antithrombotic-Drug Regimens

The three regimens were as follows: 325 mg of non-enteric-coated aspirin (Bayer, West Haven, Conn.) orally per day; 325 mg of non-enteric-coated aspirin per day and intravenous heparin (initial dose, 10,000 to 15,000 U per day), with the dose titrated to achieve an activated partial-thromboplastin time of 40 to 60 seconds and discontinued once an international normalized ratio of 2.0 to 2.5 was reached with the use of oral warfarin; and 325 mg of non-enteric-coated aspirin per day and 250 mg of ticlopidine (Ticlid, Sanofi, New York) orally twice a day. No further heparin was given after the procedure except among patients assigned to receive warfarin. The duration of ticlopidine and war-

farin treatment was four weeks. Treatment assignments were not masked, and the first dose of ticlopidine or warfarin was administered at the conclusion of the stenting procedure.

Data Collection and Analysis of End Points

Detailed case-report forms were completed by the clinical coordinator at each site, monitored by independent study monitors, and submitted to the data-coordinating center (Department of Medicine, Harvard Medical School and Beth Israel Deaconess Medical Center, Boston). Angiograms obtained during the stenting procedure were submitted to the angiographic core laboratory (Washington Hospital Center, Washington, D.C.), where they were analyzed with a computer-based system (Medis, Leiden, the Netherlands). The patients were assessed at discharge and four weeks after the stenting procedure for the occurrence of adverse clinical events. All events were classified by an independent clinical events committee whose members were unaware of the patients' treatment assignments.

The prespecified 30-day primary end point, which reflected the occurrence of stent thrombosis, was a hierarchical composite of death from any cause, revascularization of the target lesion without death, evidence of thrombosis of the target vessel on repeated angiography without revascularization, or nonfatal myocardial infarction in patients who did not undergo repeated angiography. Secondary end points included the achievement of less than 50 percent residual stenosis without death or emergency bypass surgery (defined as procedure success), procedure-related myocardial infarction, hematologic dyscrasias (neutropenia or thrombocytopenia), hemorrhagic complications, and vascular surgical complications. Myocardial infarctions (procedure-related and within 30 days after the procedure) were defined by a new Q wave that lasted at least 0.04 second in two or more contiguous leads or a creatine kinase concentration that was more than two times the upper limit of normal in the presence of an elevated concentration of MB isoenzyme. A major bleeding complication was defined as any procedure-related bleeding episode that required transfusion. Vascular surgical complications included any retroperitoneal hematoma, a vascular-access hematoma of more than 4 cm, and a pseudoaneurysm or arteriovenous fistula requiring surgery or ultrasonographic compression. Hematologic status was evaluated on the basis of a minimum of two complete blood counts performed two and four weeks after the stenting procedure; neutropenia was defined as a reduction in the absolute white-cell count to less than 1200 per cubic millimeter, and thrombocytopenia as a reduction in the platelet count to below 80,000 per cubic millimeter.

Statistical Analysis

The trial was designed to determine whether the regimen of aspirin and ticlopidine was as effective as the regimen of aspirin and warfarin in preventing stent thrombosis (the null hypothesis) and whether there were any significant differences in the primary end point between the regimen of aspirin and ticlopidine and the regimen of either aspirin and warfarin or aspirin alone. The prespecified plan therefore called for a sequential-analysis strategy. The null hypothesis was established with use of Blackwelder's formula,¹³ and two comparisons of difference in the primary end point were then made. Adjustments for multiple comparisons were made to maintain an overall type I error rate of 0.05 and are reflected in the reported P values. There was no prespecified interim analysis for early termination of the study, but the data and safety monitoring committee reviewed early safety data after the enrollment of each 250 patients.

For the null hypothesis, we assumed that the rate of stent thrombosis was 4 percent in the group assigned to aspirin and warfarin, with an increase of 3 percent or more in this value taken as an indication of the inferiority of this treatment. A total of 528 patients were required in each group for the study to have the power to detect such a difference, with a type I error of 0.05 and a type II error of 0.2. For the two conditional tests of difference,

the same 4 percent rate of stent thrombosis was assumed for the group assigned to aspirin and warfarin and the aspirin-only group. For the study to have the ability to detect a 30-day stent-thrombosis rate of 1.1 percent or less in the group assigned to ticlopidine and aspirin with a statistical power of 80 percent and a one-sided alpha error of 0.025, 527 patients were required for each group. The trial was therefore designed to enroll 550 patients per group, for a total of 1650 patients.

All comparisons were based on the intention-to-treat principle. Continuous variables were compared with the use of analysis of variance for comparisons among all three groups, and binary variables were compared with the use of the chi-square test (or Fisher's exact test in the case of any variable that included fewer than five events) and were presented with nominal two-tailed P values. Relative risks of selected primary and secondary end points were also calculated (with confidence intervals) for the prespecified pairwise comparison. Differences in the median time to a primary event were analyzed with the Kruskal-Wallis nonparametric rank-sum test for multiple groups. A stepwise multivariable logistic model of the primary end point was used to evaluate base-line predictors and the treatment effect simultaneously. All statistical analyses were performed with SAS computer software (version 6.12, SAS Institute, Cary, N.C.).¹⁴

RESULTS

Enrollment of Patients and Base-Line Characteristics

A total of 1965 patients with 2147 lesions were enrolled between February 1996 and November 1996. Of these, 1653 patients (84.1 percent) with 1772 lesions met the criteria for successful stent placement and were enrolled in the randomized trial. The remaining 312 patients with 375 lesions were enrolled in a parallel registry. Overall, 99.3 percent met the secondary end point of procedure success (less than 50 percent residual stenosis without death or emergency bypass surgery), including all 1653 patients who underwent randomization and 298 of the 312 patients (95.5 percent) entered in the parallel registry. This report focuses on the results of the randomized patients, of whom 1534 had a single lesion treated and 119 had two lesions treated. A total of 557 patients were assigned to receive aspirin alone, 550 patients were assigned to receive aspirin and warfarin, and 546 patients were assigned to receive aspirin and ticlopidine. The base-line characteristics of the patients were similar in the three groups (Tables 1 and 2). Before placement of the stent, balloon angioplasty alone was performed in 88.1 percent, rotational atherectomy in 6.2 percent, and directional atherectomy in 0.6 percent, with no pretreatment in 5.1 percent.

Primary End Point

The overall incidence of the combined primary end point was 2.3 percent, and the overall incidence of death within 30 days was 0.06 percent. The primary and secondary end points in the individual groups are summarized in Table 3, and the relative risks of selected end points are given in Table 4. The primary end point occurred in a total of 38 patients, 20 (3.6 percent) assigned to aspirin only, 15 (2.7 percent) assigned to aspirin and warfarin, and 3 (0.5 percent)

TABLE 1. BASE-LINE CHARACTERISTICS OF THE PATIENTS.*

CHARACTERISTIC	ASPIRIN ALONE (N=557)	ASPIRIN AND WARFARIN (N=550)	ASPIRIN AND TICLOPIDINE (N=546)
Age — yr	61±11	62±11	61±12
Ejection fraction — %	56±11	56±11	57±11
Female sex — no. (%)	154 (28)	163 (30)	156 (29)
Diabetes mellitus — no. (%)	99 (18)	111 (20)	99 (18)
Dyslipidemia requiring treatment — no. (%)	189 (34)	198 (36)	169 (31)
Hypertension requiring treatment — no. (%)	289 (52)	301 (55)	274 (50)
Cigarette smoking in preceding year — no. (%)	150 (27)	160 (29)	158 (29)
Single-vessel disease — no. (%)	373 (67)	369 (67)	371 (68)
Previous myocardial infarction — no. (%)	176 (32)	214 (39)	196 (36)
Angina of grade III or IV — no. (%)†	335 (60)	339 (62)	323 (59)
Previous PTCA — no. (%)	83 (15)	94 (17)	82 (15)
Previous CABG — no. (%)	44 (8)	40 (7)	41 (8)
Previous restenosis — no. (%)‡	92 (17)	98 (17)	90 (15)
Lesion grade B2 or C — no. (%)‡§	392 (66)	353 (60)	382 (65)
Angiographically evident thrombus — no. (%)‡	20 (3)	18 (3)	25 (4)
Moderate or severe calcification — no. (%)‡	133 (22)	107 (18)	122 (21)
Ostial location of lesion — no. (%)‡	42 (7)	34 (6)	37 (6)
Bifurcation — no. (%)‡	38 (6)	36 (6)	33 (6)
TIMI grade 0 flow (total occlusion) — no. (%)	11 (2)	11 (2)	10 (2)
Length of lesion — mm	10.8±5.6	10.5±5.5	10.8±5.4
Target vessel LAD — no. (%)‡	255 (43)	245 (41)	254 (44)

*Plus-minus values are means ±SD. PTCA denotes percutaneous transluminal coronary angioplasty, CABG coronary-artery bypass grafting, TIMI Thrombolysis in Myocardial Infarction, and LAD left anterior descending artery.

†The Canadian Heart Cardiovascular classification was used.

‡Not all the data were available for all the patients.

§The American College of Cardiology classification was used.

assigned to aspirin and ticlopidine ($P=0.001$ for the comparison of all three groups). The relative risk of the primary end point in the group assigned to aspirin and ticlopidine was 0.15, as compared with the risk in the group assigned to aspirin alone ($P<0.001$), and 0.20, as compared with the risk in the group assigned to aspirin and warfarin ($P=0.01$) (Table 4).

Three components of the primary end point were mainly responsible for the differences among the three groups and were highly correlated: revascularization of the target lesion ($P=0.002$), angiographically evident thrombosis ($P=0.005$), and recurrent myocardial infarction ($P=0.01$). There were also significant differences in the incidence of revascularization of the target lesion and angiographically evident thrombosis between the group assigned to aspirin

TABLE 2. ANGIOGRAPHIC AND PROCEDURAL CHARACTERISTICS.*

CHARACTERISTIC	ASPIRIN ALONE	ASPIRIN AND WARFARIN	ASPIRIN AND TICLOPIDINE
Target vessel — no. (%)	595 (34)	592 (33)	585 (33)
Dimensions of lesion before procedure			
Reference artery — mm	3.00±0.50	3.03±0.54	3.02±0.46
Minimal luminal diameter — mm	1.02±0.51	1.02±0.43	0.99±0.42
Stenosis — %†	65.8±13.1	66.1±13.5	66.9±13.0
Dimensions of lesion before stenting			
Reference artery — mm	2.98±0.51	2.99±0.52	3.00±0.48
Minimal luminal diameter — mm	1.72±0.49	1.71±0.50	1.70±0.47
Stenosis — %†	41.6±15.8	42.1±15.9	42.8±15.5
Final luminal dimensions			
Reference artery — mm	3.06±0.49	3.10±0.52	3.07±0.6
Minimal luminal diameter — mm	2.80±0.40	2.79±0.46	2.80±0.43
Stenosis — %†	7.8±12.1	9.4±11.7	8.2±11.5
Final balloon dimensions			
Mean diameter — mm	3.40±0.60	3.48±0.51	3.44±0.49
Balloon:artery ratio	1.15±0.17	1.13±0.16	1.13±0.17
Final stent dimensions — mm			
Minimal diameter	2.80±0.49	2.79±0.53	2.80±0.51
Mean diameter	3.26±0.42	3.25±0.48	3.26±0.42
Increase in diameter	1.8±0.5	1.8±0.5	1.8±0.5

*Plus-minus values are means ±SD.

†Stenosis was calculated as the average reference luminal diameter minus the minimal luminal diameter divided by the average reference luminal diameter times 100 (for the worse of two orthogonal views).

and ticlopidine and either the group assigned to aspirin only or the group assigned to aspirin and warfarin (Table 4). Among the entire randomized cohort, there was only one death, in the aspirin-alone group.

The cumulative incidence of primary events is shown in Figure 1, with a mean time to a primary event of 0.7 day for the group assigned to aspirin and ticlopidine, 2.9 days for the group assigned to aspirin and warfarin, and 3.7 days for the group assigned to aspirin alone ($P=0.17$ for the difference among the three groups). A stepwise logistic model of the primary end point was evaluated for the following potential predictors: age, sex, presence of diabetes mellitus, number of lesions treated (two vs. one), length of the lesion, dissection grade, appearance of angiographically evident thrombus, and post-treatment minimal diameter of the lumen after adjustment for the type of antithrombotic-drug regimen. The primary end point was positively associated with a dissection grade of B or greater (odds ratio, 3.82; $P=0.002$) and a smaller post-treatment minimal luminal diameter (odds ratio, 5.00 for each additional 1-mm decrease; $P<0.001$).

Among the 312 patients who did not undergo randomization, the incidence of the combined primary end point was 3.5 percent. Multivariable analysis indicated that the number of stents implanted was positively associated with stent thrombosis ($P=0.01$) and the use of aspirin and ticlopidine was negatively associated with stent thrombosis ($P=0.08$).

TABLE 3. PRIMARY AND SECONDARY EVENTS IN THE FIRST 30 DAYS AFTER STENTING.*

EVENT	ASPIRIN ALONE (N=557)	ASPIRIN AND WARFARIN (N=550)	ASPIRIN AND TICLOPIDINE (N=546)	P VALUE†
number (percent)				
Primary end point	20 (3.6)	15 (2.7)	3 (0.5)	0.001
Death	1 (0.2)	0	0	
Revascularization of target lesion	19 (3.4)	14 (2.5)	3 (0.5)	0.002
CABG	3 (0.5)	1 (0.2)	1 (0.2)	0.63
PTCA	17 (3.1)	14 (2.5)	3 (0.5)	0.003
Angiographically evident thrombosis	16 (2.9)	15 (2.7)	3 (0.5)	0.005
Recurrent myocardial infarction	15 (2.7)	11 (2.0)	3 (0.5)	0.01
Q-wave	8 (1.4)	8 (1.5)	1 (0.2)	0.04
Non-Q-wave	7 (1.3)	3 (0.5)	2 (0.4)	0.27
Other clinical events				
Procedure-related myocardial infarction	16 (2.9)	23 (4.2)	23 (4.2)	0.41
Q-wave	4 (0.7)	0	0	0.04
Non-Q-wave	12 (2.2)	23 (4.2)	23 (4.2)	0.10
Hemorrhagic complications	10 (1.8)	34 (6.2)	30 (5.5)	<0.001
Vascular surgical complications	2 (0.4)	11 (2.0)	11 (2.0)	0.02
Neutropenia or thrombocytopenia	1 (0.2)	1 (0.2)	3 (0.5)	0.46
Cerebrovascular accident	2 (0.4)	1 (0.2)	0	0.78

*CABG denotes coronary-artery bypass grafting, and PTCA percutaneous transluminal coronary angioplasty.

†The P values are for the comparison of the three groups by the chi-square test.

TABLE 4. RELATIVE RISK OF PRIMARY AND SECONDARY EVENTS IN THE GROUP ASSIGNED TO ASPIRIN AND TICLOPIDINE AS COMPARED WITH THE GROUP ASSIGNED TO ASPIRIN ALONE AND THE GROUP ASSIGNED TO ASPIRIN AND WARFARIN.*

EVENT	RELATIVE RISK (95% CI) AS COMPARED WITH ASPIRIN ALONE	P VALUE	RELATIVE RISK (95% CI) AS COMPARED WITH ASPIRIN AND WARFARIN	P VALUE
Primary end point	0.15 (0.05–0.43)	<0.001	0.20 (0.07–0.61)	0.01
Death	—	—	—	—
Revascularization of target lesion	0.16 (0.06–0.46)	0.001	0.22 (0.07–0.66)	0.02
Angiographically evident thrombosis	0.19 (0.06–0.57)	0.001	0.20 (0.07–0.61)	0.01
Recurrent myocardial infarction	0.20 (0.07–0.62)	0.014	0.27 (0.08–0.90)	0.11
Neutropenia or thrombocytopenia	3.06 (0.36–26.2)	0.74	3.02 (0.35–25.91)	0.75
Hemorrhagic complications	3.06 (1.57–5.97)	0.002	0.88 (0.55–1.43)	0.99
Vascular surgical complications	5.61 (1.49–21.16)	0.02	1.01 (0.44–2.30)	0.99

*CI denotes confidence interval.

Secondary End Points

There were no significant differences in the incidence of procedure-related myocardial infarctions among the three groups (overall incidence, 3.8 percent) (Table 3). There were three cerebrovascular accidents (overall incidence, 0.2 percent) and five cases of severe neutropenia or thrombocytopenia (overall incidence, 0.3 percent). The incidence of hemorrhagic complications and vascular surgical complications was significantly different among the three groups ($P < 0.001$ and $P = 0.02$, respectively) (Table 3). The group assigned to aspirin and ticlopidine had a relative risk of hemorrhagic complications of 3.06 ($P = 0.002$) and a relative risk of vascular surgical complications

of 5.61 ($P = 0.02$) as compared with the group assigned to aspirin alone. The risk of these events in the group assigned to aspirin and ticlopidine was similar to that in the group assigned to aspirin and warfarin.

DISCUSSION

This randomized trial compared the ability of three antithrombotic-drug regimens to prevent stent thrombosis. The chief finding was that a combination of aspirin and ticlopidine was superior to either a combination of warfarin and aspirin or aspirin alone in the prevention of stent thrombosis after successful stenting. The risk of stent thrombosis with aspirin and warfarin was slightly lower than with aspirin alone.

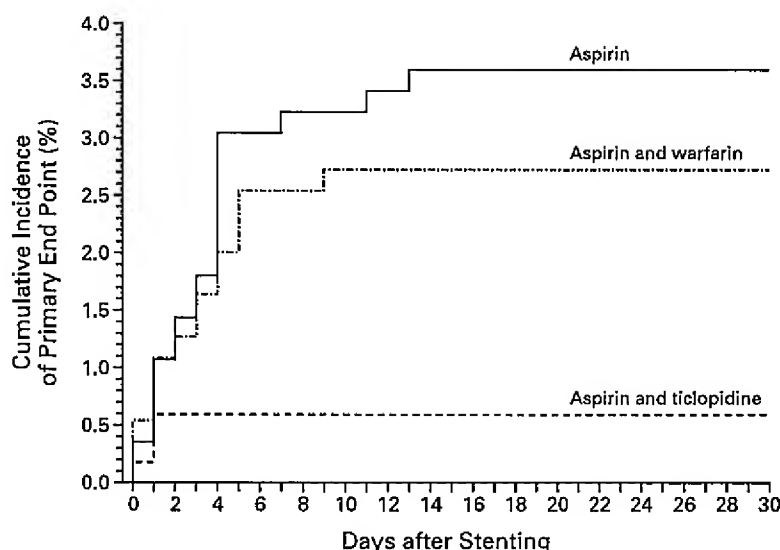


Figure 1. Cumulative Incidence of the Primary End Point in the Three Treatment Groups.

Our study differs from earlier trials in that randomization required high stent-implantation pressures and the achievement of a predefined optimal result. Our finding of an intermediate (2.7 percent) risk of stent thrombosis among patients assigned to receive aspirin and warfarin, between that of the patients assigned to receive aspirin and ticlopidine (0.5 percent) and that of the patients assigned to receive aspirin alone (3.6 percent), is in contradistinction to earlier data from a single-center randomized trial conducted in Germany, which suggested that warfarin may promote stent thrombosis.⁹ Several factors may explain this difference: the intensity of anticoagulation was lower in our study (target international normalized ratio, 2.0 to 2.5), the sample population in the German study included many patients with acute myocardial infarction (24 percent), and our patients had to have a successful angiographic result before they underwent randomization. The modified regimen of aspirin and warfarin that was used in our study was at least as effective as aspirin alone in preventing stent thrombosis, with no added risk of stent thrombosis. The high rate of stent thrombosis in the group assigned to aspirin alone is an important finding and contradicts the results of a recent registry study and of a small, randomized trial, which reported that the rate of stent thrombosis was similar in the group assigned to aspirin and ticlopidine and the group treated with aspirin alone.^{11,12}

Our study has several important secondary findings. The overall death rate was 0.06 percent, and only 1 of 38 patients (2.6 percent) who reached the primary end point died. This rate is markedly lower than the rate of death from stent thrombosis in previous trials (range, 11 to 24 percent^{1-3,9,15,16}) and may reflect differences in selection factors used or improved diagnosis and treatment strategies for stent thrombosis. The fact that Q-wave myocardial infarction occurred in 45 percent of those who reached the primary end point in our study is evidence that the clinical consequences of stent thrombosis remain severe.

The significantly lower incidence of stent thrombosis during treatment with aspirin and ticlopidine as compared with treatment with aspirin alone was offset by a slight but significantly increased risk of hemorrhagic and vascular surgical complications. Despite the widespread belief that combination therapy with aspirin and ticlopidine is associated with a lower rate of vascular surgical complications than therapy with aspirin and warfarin, there was no significant difference in these end points between the two groups. Interestingly, the incidence of hemorrhagic complications in the group assigned to aspirin and warfarin was lower in our study than in previous trials,^{2,3} suggesting that femoral-artery puncture and sheath-removal techniques have improved over the years.

There was also no significant difference in the risk of neutropenia or thrombocytopenia between the

group assigned to aspirin and ticlopidine (0.5 percent) and either the group assigned to aspirin alone (0.2 percent) or the group assigned to aspirin and warfarin (0.2 percent). These values are similar to the 0.8 percent incidence of severe neutropenia more than two months after the start of treatment reported for patients who receive long-term ticlopidine therapy to prevent strokes.¹⁰ An incidence of reversible ticlopidine-induced neutropenia of 0.8 percent was also reported in the French Multicenter Registry of 2900 patients.¹⁶ Although these data may be taken as evidence that it is safe to use ticlopidine for up to 4 weeks, stent thrombosis generally occurs within the first 14 days after the stenting procedure and may occur even earlier (a mean of 0.7 day in our study) in patients who are given aspirin and ticlopidine. Although not directly evaluated in this study, the early time to thrombosis indirectly suggests that the course of ticlopidine may be shortened to two weeks, which may further reduce the risk of neutropenia and thrombocytopenia. Importantly, despite the absence of severe hematologic dyscrasias in our study, there are continued reports of thrombotic thrombocytopenic purpura in association with ticlopidine therapy (incidence, approximately 1 in 1600 patients), including short-term treatment after coronary stenting.^{17,18} The vast majority of these hematologic disturbances occur after two weeks of ticlopidine treatment,¹⁸ thereby lending further support to the suggestion to shorten the duration of ticlopidine therapy after stenting to two weeks.

In summary, we demonstrated the efficacy and safety of a combination of ticlopidine and aspirin as a means of preventing stent thrombosis in patients with ischemic coronary syndromes. The superiority of this antithrombotic regimen over aspirin alone and a combination of aspirin and warfarin supports its use as the primary therapy after coronary stenting.

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APPENDIX

The following principal investigators and study coordinators also participated in the Stent Anticoagulation Restenosis Study: *University of Texas Health Science Center—Brooke Army Medical Center, San Antonio:* S. Bailey, G. Cooper-Read; *Beth Israel Deaconess Medical Center, Boston:* P. Rooney; *Scripps Memorial Hospital, La Jolla, Calif.:* M. Buchbinder, D. Koester; *Lancaster Heart Foundation, Lancaster, Pa.:* P. Casale, J. Tuzi; *Yale University Hospital, New Haven, Conn.:* M. Cleman, J. Davey; *Northwestern University Medical School, Chicago:* C. Davidson, J. Espisito; *Baylor University Medical Center, Dallas:* S. DeMaio, I. Joukova; *Temple University, Philadelphia:* E. Deutsch, B. Flynn; *Georgetown University, Washington, D.C.:* J. Gannuscio; *Cleveland Clinic Foundation, Cleveland:* S. Ellis, L. Krcuska; *Texas Heart Institute, Houston:* D. Fish, M. Harlan; *Aurora Denver Cardiology, Aurora, Colo.:* S. Friedrich, K. Bickett; *Mid-West Cardiology Research Foundation, Columbus, Ohio:* B. George, D. Smith; *St. Joseph's Hospital, Syracuse, N.Y.:* S. Wagner; *Miriam Hospital, Providence, R.I.:* N. Wright; *Arizona Heart Institute, Phoenix:* R. Heuser, S. Spooner; *University of Pennsylvania, Philadelphia:* J. Hirshfeld, L. Felderstein; *Mayo Clinic, Rochester, Minn.:* D. Holmes, L. Pierre; *Christ Hospital, Cincinnati:* D. Kerciakes, B. Gervais, L. Martin; *Atlanta Cardiology, Atlanta:* W. Knopf, N. Yarbrough; *Washington Hospital Center, Washington, D.C.:* K. Donovan; *Hartford Hospital,*

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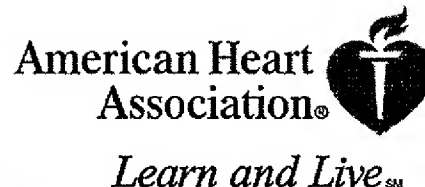
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Exhibit 18

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Double-Blind Study of the Safety of Clopidogrel With and Without a Loading Dose in Combination With Aspirin Compared With Ticlopidine in Combination With Aspirin After Coronary Stenting : The Clopidogrel Aspirin Stent International Cooperative Study (CLASSICS)

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for the CLASSICS Investigators

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Double-Blind Study of the Safety of Clopidogrel With and Without a Loading Dose in Combination With Aspirin Compared With Ticlopidine in Combination With Aspirin After Coronary Stenting

The Clopidogrel Aspirin Stent International Cooperative Study (CLASSICS)

Michel E. Bertrand, MD; Hans-Jürgen Rupprecht, MD; Philip Urban, MD; Anthony H. Gershlick, MD;
for the CLASSICS Investigators

Background—Combination therapy with the ADP receptor antagonist ticlopidine plus aspirin has emerged as standard care after coronary stenting. Clopidogrel, a new ADP receptor antagonist, has greater molar potency than ticlopidine and better safety/tolerability.

Methods and Results—Patients (n=1020) were randomized after successful stent placement and initiated on a 28-day regimen of either (1) 300-mg clopidogrel loading dose and 325 mg/d aspirin on day 1, followed by 75 mg/d clopidogrel and 325 mg/d aspirin; (2) 75 mg/d clopidogrel and 325 mg/d aspirin; or (3) 250 mg BID ticlopidine and 325 mg/d aspirin. The primary end point consisted of major peripheral or bleeding complications, neutropenia, thrombocytopenia, or early discontinuation of study drug as the result of a noncardiac adverse event during the study-drug treatment period. The primary end point occurred in 9.1% of patients (n=31) in the ticlopidine group and 4.6% of patients (n=31) in the combined clopidogrel group (relative risk 0.50; 95% CI 0.31 to 0.81; $P=0.005$). Overall rates of major adverse cardiac events (cardiac death, myocardial infarction, target lesion revascularization) were low and comparable between treatment groups (0.9% with ticlopidine, 1.5% with 75 mg/d clopidogrel, 1.2% with the clopidogrel loading dose; $P=NS$ for all comparisons).

Conclusions—The safety/tolerability of clopidogrel (plus aspirin) is superior to that of ticlopidine (plus aspirin) ($P=0.005$). The 300-mg loading dose was well tolerated, notably with no increased risk of bleeding. Secondary end point data are consistent with the hypothesis that clopidogrel and ticlopidine have comparable efficacy with regard to cardiac events after successful stenting. (*Circulation*. 2000;102:624-629.)

Key Words: aspirin ■ receptors ■ stents ■ thrombosis ■ ticlopidine

Intracoronary stenting is widely used to treat vessel closure after PTCA or electively during angioplasty to decrease the rate of restenosis.¹ Current stents are metallic and thrombogenic, resulting in a risk of acute and subacute thrombosis within the first month after stent placement.² Such thrombotic events result in serious clinical consequences, including death, myocardial infarction (MI) or emergency CABG. Initial attempts to reduce stent thrombosis involved regimens combining heparin, oral anticoagulant, and aspirin, but these were hampered by a high rate of complications, especially bleeding requiring blood transfusion and puncture site complications requiring surgical repair.³⁻⁵

Several randomized trials have shown that combination therapy with aspirin plus ticlopidine is superior to heparin and coumarin in preventing subacute stent occlusion.⁶⁻⁹ These benefits have been demonstrated prospectively in low-risk,⁶ mixed-risk or intermediate-risk,^{6,7} and high-risk patients.⁹ The ticlopidine-aspirin combination leads to fewer hemorrhagic or peripheral complications than the conventional regimen combining oral anticoagulant with aspirin (0% to 2% versus 3% to 7%). Moreover, the dual antiplatelet approach shows better efficacy than aspirin alone.^{8,10} Thus, the combination of 250 mg BID ticlopidine and aspirin has become the reference antithrombotic therapy after coronary stenting,

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A list of all CLASSICS participants is given in the Appendix.

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although full antiplatelet effect requires a few days because of the delayed onset of action by ticlopidine.¹¹

Clopidogrel (Plavix/Iscover), a new platelet ADP receptor antagonist, has a more potent platelet antiaggregant effect than ticlopidine,¹² a faster onset of action, and does not cause the adverse events that limit ticlopidine therapy.¹³ A loading dose of clopidogrel produces rapid and pronounced diminution of 5 $\mu\text{mol/L}$ ADP-induced platelet aggregation in human volunteers.¹⁴ This suggests the potential for an earlier therapeutic benefit in the prevention of stent thrombosis. A preclinical study showed that when aspirin is combined with acute high-dose or chronic low-dose clopidogrel, graft and stent thrombosis is significantly reduced in a synergistic manner.¹⁵ Makkar et al¹⁶ have provided further preclinical evidence for synergism between clopidogrel and aspirin. These findings predict that concurrent inhibition of the ADP and thromboxane A_2 pathways of platelet recruitment will produce additive and/or synergistic decreases in thrombo-occlusive events. We therefore evaluated the safety of clopidogrel (with or without a loading dose) in combination with aspirin compared with ticlopidine in combination with aspirin in patients who had undergone successful coronary stenting.

Methods

Objectives

The primary objective was to evaluate, for the treatment period, the relative safety of clopidogrel (with or without an initial loading dose) plus aspirin compared with ticlopidine plus aspirin in patients who had undergone successful intracoronary stenting. The secondary objective was to evaluate the incidence occurrence of cardiac events during the period of study drug administration.

Study Design

Forty-eight centers in 8 European countries enrolled patients between May and November 1998. The study was designed as a multicenter, randomized, controlled, double-blind, parallel-group trial. Written informed consent was obtained from each patient, and the study was performed according to local regulations, the principles of the Helsinki Declaration, and the *European Guidelines for Good Clinical Practice*.

Randomization

After coronary stenting, and on receipt of informed consent and satisfactory documentation of all inclusion and exclusion criteria, eligible patients were randomized into 1 of 3 treatment groups.

Inclusion and Exclusion Criteria

Randomized patients satisfied the following criteria: successful planned or unplanned coronary stenting (1 or 2 stents) in a single vessel (reference vessel diameter >2.8 mm) with the use of any commercially available non-heparin-coated stents; $<10\%$ adjacent residual stenosis; no angiographic evidence of thrombus formation or dissection within the treated vessel; blood flow of TIMI grade 3 in each stented segment and associated major side branches; preoperative creatine phosphokinase (CPK) levels less than twice the upper limit of normal (ULN); and eligibility to commence study drug within 6 hours after stent implantation. Principal exclusion criteria were stenting procedure involving \geq stents or >1 vessel, involving the left main coronary artery or a major bifurcation, or involving vein grafts; primary angioplasty for ongoing myocardial infarction with documented ST-segment elevation and/or elevated CPK-MB levels $>2\times$ ULN and CPK MB levels greater than normal; persistent objective ischemia determined by 12-lead ECG between stenting and randomization; administration of oral anticoagulants, GP IIb/IIIa receptor antagonists and other antiplatelet agents, except for aspirin,

within 1 month before randomization; administration of thrombolytics 2 weeks before randomization; need for anticoagulants, thrombolytic agents, or GP IIb/IIIa receptor antagonists after the procedure; percutaneous or surgical revascularization (PTCA, CABG) within 2 months before the procedure; history of allergy or intolerance or contraindication to aspirin, ticlopidine, or clopidogrel.

Study Drugs and Procedures

All study drugs (including aspirin) were administered on a blinded basis (double-dummy) and were to be initiated within 6 hours of completion of stenting. Patients were to receive 28 days of treatment with either (1) 300 mg clopidogrel (loading dose) and 325 mg/d aspirin on day 1, followed by 75 mg/d clopidogrel and 325 mg/d aspirin (days 2 to 28); (2) 75 mg/d clopidogrel and 325 mg/d aspirin (days 1 to 28); (3) 250 mg BID ticlopidine and 325 mg/d aspirin (days 1 to 28).

Heparin was discontinued at the end of the procedure and 4 hours before sheath removal. In cases in which stent placement was performed in the late afternoon, intravenous heparin could be continued for a few hours to avoid sheath removal during the night, provided that the total duration of administration did not exceed 36 hours.

End Points

A Critical Event Adjudication Committee validated all potential outcome events; only validated events were analyzed. The primary end point was the incidence of any one of the following validated events occurring during the study drug treatment period between visits 1 and 4 or until discontinuation of study drug: (1) major peripheral or bleeding complications (including false aneurysms, surgical repair of puncture site complications, blood transfusion ≥ 2 U of blood), intracranial bleeding, retroperitoneal bleeding, overt hemorrhage with a decrease of hemoglobin ≥ 3 g/dL compared with baseline); (2) neutropenia (neutrophil count $<1.5\times 10^9/L$); (3) thrombocytopenia (platelet count $<100\times 10^9/L$); (4) early discontinuation of study drug because of a noncardiac adverse event (including death of noncardiac origin).

Secondary evaluation criteria for safety were incidence of the specific adverse events rash or urticaria, pruritus, and diarrhea; incidence of any adverse event or other specific groups of adverse events; change from baseline to visit 4 in laboratory parameters.

The secondary (efficacy) end points were the incidence of the following events during the treatment period: (1) cardiac events (combined and separately): cardiovascular death (including all deaths not definitively ascribed to a specific noncardiac cause); MI (spontaneously or in association with angioplasty or CABG); or target vessel revascularization (performed because of recurrent ischemia, arrhythmia, or hemodynamic failure).

MI occurring in the absence of angioplasty or CABG was diagnosed by new abnormal Q waves not present at baseline or CPK levels increased beyond $2\times$ ULN, together with a CPK-MB increase above the ULN and/or measurements of troponin T >0.2 $\mu\text{g/L}$. After angioplasty, CPK and CPK-MB had to be $>3\times$ ULN and/or troponin T >1.0 $\mu\text{g/L}$. After CABG, CPK and CPK-MB had to be $>5\times$ ULN and/or troponin T had to be >2.0 $\mu\text{g/L}$.

Statistical Methods

Study Power

The incidence of the primary event cluster for the clopidogrel arm was projected from the Clopidogrel versus Aspirin in Patients at Risk of Ischaemic Events (CAPRIE) study,¹³ in which the incidence of discontinuations of study drug as the result of noncardiac adverse events during the first 28 days of treatment was 2.5% and the incidence of neutropenia and thrombocytopenia were each $<0.1\%$. On the basis of data available for ticlopidine in stent patients, it was reasonable to predict that the primary event rate including bleeding would be between 2.5% and 5% in the clopidogrel group. Power calculations were based on the assumption that the event rate for ticlopidine would be 5% greater than for clopidogrel with the use of a sample size of 335 per group (670 for the pooled clopidogrel

TABLE 1. Baseline Clinical Characteristics

	Total (n=1020)	Ticlopidine 250 mg BID (n=340)	Clopidogrel 75 mg QD (n=335)	Clopidogrel 300/75 mg QD (n=345)	P
Mean age \pm SD, y	60 \pm 10.1	61 \pm 9.9	60 \pm 10.4	60 \pm 10.1	0.757
Sex, M/F, %	77/23	75/25	78/22	77/23	0.557
Previous unstable angina, n (%)	441 (43.2)	154 (45.3)	132 (39.4)	155 (44.9)	0.224
Previous stable angina, n (%)	569 (55.8)	177 (52.1)	201 (60.0)	191 (55.4)	0.114
Silent ischemia, n (%)	75 (7.4)	27 (7.9)	30 (9.0)	18 (5.2)	0.154
Previous MI, n (%)	370 (36.3)	123 (36.2)	121 (36.1)	126 (36.5)	0.993
Treatment for diabetes, n (%)	115 (11.3)	41 (12.1)	35 (10.4)	39 (11.3)	0.803
Hypertension, n (%)	509 (49.9)	165 (48.5)	173 (51.6)	171 (49.6)	0.713
Treatment for hypercholesterolemia, n (%)	581 (57.0)	199 (58.5)	187 (55.8)	195 (56.5)	0.761
Former or current smoker, n (%)	704 (69.0)	225 (66.2)	237 (70.7)	242 (70.1)	0.396

groups). For an event rate of 2.5% in the clopidogrel group, the study would have 92% power to detect a significant difference between clopidogrel and ticlopidine at the 5% significance level (for the pooled clopidogrel groups) and would have 74% power to detect a significant difference at the 2.5% level (for the separate clopidogrel groups). For a clopidogrel event rate of 5%, the corresponding values were 79% and 54%.

Primary End Point

Assessment of relative safety was based on a comparison between treatment groups of the proportions of patients who had a primary end point event(s). Proportions were compared between treatment groups by means of Fisher's exact test (2-sided). On the basis of prospective decision rules given in the study protocol, the 2 clopidogrel groups were pooled and compared with ticlopidine at the 5% significance level. The 2 clopidogrel groups were also compared at the 5% level. Because there was a significant difference between the clopidogrel groups, separate comparisons of each clopidogrel regimen to ticlopidine were also performed, based on prospective decision rules. Bonferroni adjustment was performed when testing of each clopidogrel group to ticlopidine was indicated. Estimates and 95% confidence intervals for the relative risk of an event were calculated for pairs of treatments.

Secondary End Points

Assessments of efficacy, based on the cardiac and death end points, were carried out in the same manner as for the primary end point.

Additional safety assessments were based on the proportion of patients with ≥ 1 episodes of a specific adverse event or groups of events and the change from baseline to day 28 for each laboratory parameter. The same testing strategy as used for the primary end point was applied. The proportions of events in the treatment groups were compared by means of Fisher's exact test (2-sided), and laboratory changes from baseline were compared between treatments with the use of a 1-way ANOVA.

Only events occurring during the treatment period (from randomization to the day after the last dose of study drug) were included in the primary analysis.

Results

One thousand twenty-one patients were enrolled in 48 centers from 8 European countries (see the Appendix). One randomized patient withdrew consent immediately before taking his first study medication. Baseline characteristics for the 1020 patients who received study drug are given in Table 1; Table

TABLE 2. Stent Procedural Details

Parameter	Total (n=1020)	Ticlopidine 250 mg BID (n=340)	Clopidogrel 75 mg QD (n=335)	Clopidogrel 300/75 mg QD (n=345)
Balloon angioplasty, %	95.2	95.9	96.4	93.3
Stented segment, %*				
LAD	45.6	46.9	43.0	47.0
LCx	19.6	16.2	23.9	18.8
RCA	33.4	35.7	31.9	32.5
Stents implanted, %†				
1	87.5	84.4	89.3	88.7
2	12.3	15	10.5	11.3
Time from stent to start of dosing, mean \pm SD, h‡	2.4 \pm 1.5	2.4 \pm 1.5	2.3 \pm 1.5	2.4 \pm 1.6

LAD indicates left anterior descending; LCx, left circumflex; and RCA, right coronary artery.

Several patients were protocol deviators with respect to the stent procedure: *13 patients (1.3%) were stented in the left main stem, and 1 patient (0.1%) received stents in more than 1 vessel; †3 patients (0.3%) received ≥ 3 stents; ‡10 patients (1.0%) had stent-dosing times that exceeded 6 hours. Data for time from stent to start of dosing were available for 337 of 340 patients in the ticlopidine group, 331 of 335 patients in the clopidogrel 75 mg QD group, and 344 of 345 patients in the clopidogrel 300/75 mg group.

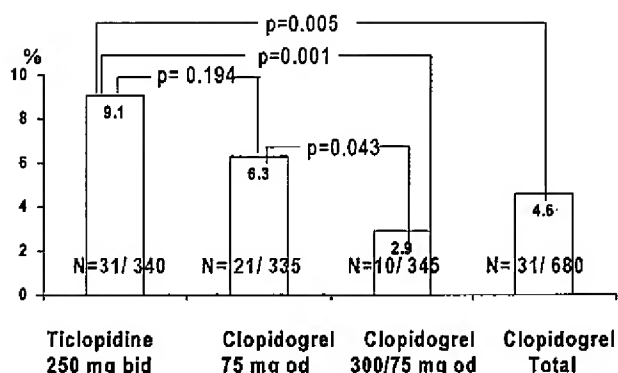


Figure 1. Occurrence of primary end point by treatment.

2 gives details on stent implantation. There were no significant differences between treatment groups.

Primary Study End Point

Figure 1 summarizes data on the primary end point at 28 days, which occurred in 9.1% (31 of 340) of patients in the ticlopidine group and in 4.6% (31 of 680) of patients in the combined clopidogrel group—a relative risk reduction of 50% (95% CI 31% to 81%; $P=0.005$) in favor of clopidogrel. The incidence of the primary end point at 28 days was 6.3% (21 patients) in the 75 mg QD clopidogrel group and 2.9% (10 patients) in the clopidogrel loading-dose group ($P=0.043$).

Table 3 provides a breakdown of primary end point data. The incidence of major peripheral or bleeding complications was low and similar in the 3 groups (1.2% for ticlopidine, 1.2% for 75 mg QD clopidogrel, and 1.5% for clopidogrel loading dose) during the treatment period. The risk of an event in the clopidogrel loading-dose group was approximately a third of that for ticlopidine patients (2.9% versus

9.1%). This was primarily due to a higher frequency of discontinuations as the result of noncardiac adverse events (8.2% with ticlopidine versus 2.0% with the clopidogrel loading dose), which, in turn, was due to an increased frequency of discontinuations because of skin disorders (mostly rash) (2.6% with ticlopidine versus 0.6% with the clopidogrel loading dose), gastrointestinal disturbances (2.6% versus 0.3%), and allergic adverse events (1.2% versus 0%).

One (0.3%) ticlopidine patient developed neutropenia (neutrophil count $<0.1 \times 10^9/L$) 28 days after randomization and recovered without sequelae 7 days after the end of treatment. Two patients (0.6%) in each clopidogrel group had mild thrombocytopenia (70 to $100 \times 10^9/L$), although these cases were transient and without clinical significance. In 3 of the cases, heparin was given concomitantly and there was no premature treatment cessation.

Secondary End Points

There was a low and comparable overall rate of major adverse clinical events (MACE) in the 3 groups: 0.9% with ticlopidine, 1.2% with the clopidogrel loading dose, and 1.5% with 75 mg QD clopidogrel. There were no statistically significant differences between the combined clopidogrel group and ticlopidine ($P \geq 0.555$) or between the 2 clopidogrel groups ($P \geq 0.058$) for any of the secondary end points (see Table 4).

Analyses of the primary and secondary end points with all the validated outcome events recorded in the study, including those occurring during the follow-up period, did not change the overall conclusion.

Discussion

The CLOpidogrel ASpirin Stent International Cooperative Study (CLASSICS) is the first randomized trial of clopidogrel in coronary stenting and the first to evaluate

TABLE 3. Summary of Data on Primary (Safety) End Point

Outcome Event	Ticlopidine (n=340)	Clopidogrel 75 mg QD (n=335)	Clopidogrel 300/75 mg QD (n=345)	Clopidogrel Total (n=680)
Composite (any of the below)*	31 (9.1%)	21 (6.3%)	10 (2.9%)	31 (4.6%)
Major peripheral or bleeding complication	4 (1.2%)	4 (1.2%)	5 (1.5%)	9 (1.3%)
Neutropenia $<1.5 \times 10^9/L$	1 (0.3%)	0	0	0
Thrombocytopenia 70–100 $\times 10^9/L$	0	2 (0.6%)	2 (0.6%)	4 (0.6%)
Early discontinuation due to noncardiac event	28 (8.2%)	17 (5.1%)	7 (2%)	24 (3.5%)
Skin disorder	9 (2.6%)	3 (0.9%)	2 (0.6%)	5 (0.7%)
Gastrointestinal disorder	9 (2.6%)	8 (2.4%)	1 (0.3%)	9 (1.3%)
Allergy	4 (1.2%)	0	0	0
Others	6 (1.8%)	6 (1.8%)	4 (1.2%)	10 (1.5%)

*Patients may have had >1 of the events included in the end point. Breakdown of discontinuations is based on the primary reason, for example, if a patient had multiple reasons for early discontinuation, they were included in one of the specific categories if possible rather than "other" or the specific category and other. If they did not have a reason that fell into one of the specific categories, they were included under "other." No patient had reasons that fell into 2 specific categories.

For frequency of composite outcome events: $P=0.005$ for ticlopidine vs clopidogrel combined; $P=0.001$ for ticlopidine vs 300/75 mg QD clopidogrel; $P=0.194$ for ticlopidine vs 75 mg QD clopidogrel; $P=0.043$ for 300/75 mg QD clopidogrel vs 75 mg QD clopidogrel.

TABLE 4. Summary of Data on Secondary (Efficacy) End Point

	Ticlopidine (n=340)	Clopidogrel 75 mg QD (n=335)	Clopidogrel 300/75 QD (n=345)
No. of patients with ≥1 cardiac event*	3 (0.9%)	5 (1.5%)	4 (1.2%)
Details	1 MI 1 MI+TLR 1 TLR	1 MI 3 MI+TLR 1 TLR	2 MI 1 Fatal MI 1 SD

TLR indicates target lesion revascularization; SD, sudden death.

*P=NS for all comparisons.

clopidogrel-aspirin combination therapy and a loading dose of clopidogrel. The rationale for CLASSICS stemmed from (1) clear evidence from the Intracoronary Stenting and Antithrombotic Regimen trial (ISAR), the Full Anticoagulation Versus Aspirin and Ticlopidine trial (FANTASTIC), the Multicenter Aspirin and Ticlopidine Trial after Intracoronary Stenting (MATTIS), and the Stent Anti-thrombotic Regimen Study (STARS) that the ticlopidine-aspirin combination improves clinical outcome after stent implantation, compared with aspirin alone or aspirin plus full anticoagulation with heparin and coumarin⁶⁻⁹; (2) the safety profile of ticlopidine, which may result in early discontinuation of the drug; (3) the superior safety profile of clopidogrel compared with ticlopidine; and (4) the comparable clinical efficacy of these 2 ADP receptor antagonists.^{13,17} The decision to test a clopidogrel loading dose was based on data from healthy volunteers,¹⁸ which showed that a loading dose produced a faster onset of platelet inhibition. The 300-mg loading dose was chosen to provide an optimal benefit/risk ratio.

Because of limited information on the use of clopidogrel and aspirin in combination, CLASSICS was primarily a safety study, with a primary end point consisting of major peripheral or bleeding complications, neutropenia, thrombocytopenia, or early discontinuation of the study drug for noncardiac adverse events. The primary end point occurred in a higher percentage of patients in the ticlopidine group (9.1%) than in the combined clopidogrel group (4.6%) ($P=0.005$), demonstrating a superior safety profile for clopidogrel. The safety advantage of clopidogrel is derived from a lower frequency of noncardiac adverse events, with significantly fewer cases of skin disorders (0.7% versus 2.6%), gastrointestinal disorders (1.3% versus 2.6%), and allergy (0% versus 1.2%). These differences indicate that with clopidogrel, more patients will be able to benefit from a full course of therapy with an effective combination antiplatelet regimen, and thus the risk of subacute stent thrombosis caused by early discontinuation of ticlopidine should be reduced.

Data from CLASSICS are supported by findings from a nonrandomized comparison of combination therapy in coronary stent patients. Moussa et al¹⁹ compared the safety and effectiveness of clopidogrel and aspirin with those of ticlopidine and aspirin in a consecutive series of patients ($n=1406$ for ticlopidine; $n=238$ for clopidogrel). At 1-month follow-up, no difference was found in the rates of stent thrombosis or MACE between the 2 groups. No clopidogrel-treated patient had neutropenia, and there was a significantly lower overall

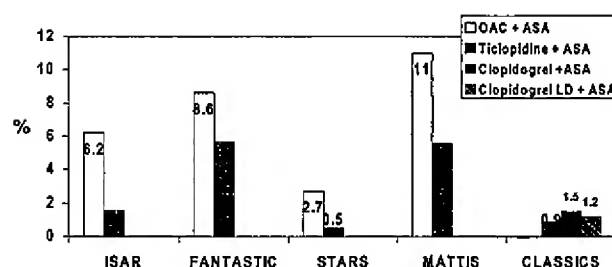


Figure 2. Comparison of MACE rates (%) in CLASSICS with those of ISAR,⁶ FANTASTIC,⁷ STARS,⁸ and MATTIS⁹ trials. OAC indicates oral anticoagulants; ASA, acetylsalicylic acid (aspirin); and LD, loading dose.

incidence of medication side effects (neutropenia, diarrhea, rash) with clopidogrel compared with ticlopidine.

Comparison of incidence rates for MACE (death, MI, revascularization) in CLASSICS with those in the ISAR, FANTASTIC, MATTIS, and STARS trials reveals that the event rates in all 3 arms of CLASSICS were lower than those in the ticlopidine-aspirin arms of FANTASTIC and MATTIS and comparable to those reported for ticlopidine plus aspirin in ISAR and STARS (Figure 2). These data reinforce the superiority of the ADP receptor antagonist-aspirin combination in improving clinical outcome after coronary stent placement.

No rebound phenomenon was observed in this study, as in the previous trials. This could be potentially of interest in special situations such as after brachytherapy, in which cases of late stent occlusion have been described.

Results from CLASSICS should be viewed in the context of the study design. First, patients were randomized after successful stenting and were therefore a relatively low-risk population. Second, although the incidence of MACE was low and similar in the 3 treatment arms, this trial was underpowered to show efficacy differences. Third, administration of GP IIb/IIIa receptor antagonists in the month before randomization or after stenting were exclusion criteria; therefore, CLASSICS does not provide information on concomitant use of clopidogrel with these agents.

Conclusions

The safety/tolerability of clopidogrel (plus aspirin) is superior to that of ticlopidine (plus aspirin). The 300-mg loading dose was well tolerated, notably with no increased risk of bleeding. Secondary end point data are consistent with the hypothesis that clopidogrel and ticlopidine have comparable efficacy regarding cardiac events after successful stent placement; however, the study was not powered to draw definitive conclusions on efficacy. The favorable benefit/risk ratio of clopidogrel and aspirin, including the use of a loading dose, supports their combined use in coronary stent patients.

Appendix

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Exhibit 19

The Clopidogrel in Unstable angina to prevent Recurrent Events (CURE) trial programme

Rationale, design and baseline characteristics including a meta-analysis of the effects of thienopyridines in vascular disease

CURE Study Investigators*

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Background Other than aspirin, there are few oral anti-thrombotic treatments with proven efficacy in patients with acute coronary syndrome. In this report, we present the rationale, design and baseline characteristics of the Clopidogrel in Unstable angina to prevent Recurrent ischaemic Events (CURE) trial, which includes a meta-analysis of the effects of thienopyridines in patients with vascular disease.

Methods and Results Combined data from randomized trials of thienopyridines in patients with atherosclerotic disease demonstrated a 29% reduction in vascular events when compared with placebo/control ($n=2392$) (OR 0.71, 95% CI 0.58–0.86, $P=0.0006$) and a 10% reduction in vascular events when compared with aspirin ($n=22\,254$) (OR 0.91, 95% CI 0.84–0.99, $P=0.039$). Similarly, randomized trials of aspirin plus thienopyridines in patients undergoing intracoronary stenting, demonstrated a marked benefit of aspirin plus ticlopidine in reducing death or myocardial infarction compared with aspirin alone (OR 0.23, 95% CI 0.11–0.49, $P=0.0001$) or aspirin plus warfarin (OR 0.51, 95% CI 0.33–0.78, $P=0.002$). Whether these benefits extend to the much larger population of patients with acute coronary syndrome is unknown. CURE is an international, randomized, double-blind trial, in which patients with acute coronary syndrome will be randomized to receive either a bolus dose of clopidogrel (300 mg) followed by 75 mg per day for 3–12 months, or matching placebo. Both groups will

receive aspirin. The co-primary efficacy end-points of CURE are: (1) the composite of cardiovascular death, myocardial infarction or stroke; and (2) the composite of cardiovascular death, myocardial infarction, stroke or refractory ischaemia. CURE will recruit approximately 12 500 patients with acute coronary syndrome (from 28 countries) and its power to detect moderate treatment benefits will be in the region of 80–90%, while maintaining an overall type I error (α) of 0.05. The baseline characteristics of the study population are consistent with at least a moderate risk group of patients with acute coronary syndrome.

Conclusions Randomized trials of thienopyridines in patients with vascular disease demonstrate that thienopyridines are effective in reducing vascular events when compared with placebo/control or aspirin, as well as when used in combination with aspirin in patients undergoing intracoronary stent implantation. The CURE trial is a large international study to determine if acute and long-term treatment with the combination of clopidogrel and aspirin is superior to aspirin alone in patients with acute coronary syndrome.

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Key Words: Thienopyridines, unstable angina, meta-analysis, clinical trial.

Introduction

Atherosclerotic plaque rupture, erosion or disruption with superimposed thrombus formation ('athero-

thrombosis) is the most important underlying pathological mechanism to cause acute coronary syndromes (unstable angina and non-Q wave infarction)^[1]. Despite treatment with aspirin and intravenous heparin, the incidence of cardiovascular death and new myocardial infarction remain substantial at 6–8% during the acute phase of hospitalization. In addition, the incidence

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of these events remains high during long-term follow-up, at 6–8% per year over at least the next 2 years^[2]. Therefore, there is a need to develop strategies to reduce both short- and long-term risks of cardiovascular events^[3]. While newer agents, such as intravenous glycoprotein (GP) IIb/IIIa receptor antagonists in addition to unfractionated heparin^[4,5], reduced events when given for a short period of time in the acute phase, longer-term oral administration of these agents has failed to demonstrate a reduction in events^[6,7]. Similarly, long-term treatment with low molecular weight heparins beyond the first week has not been beneficial, but substantially increases bleeding^[8,9]. The failures of long-term oral GP IIb/IIIa receptor antagonists or low molecular weight heparin to provide additional benefit emphasize the need to develop alternative strategies to decrease both early and late events in patients with acute coronary syndrome.

The thienopyridines

The thienopyridines, ticlopidine and clopidogrel, are antiplatelet agents that inhibit platelet aggregation induced by adenosine diphosphate (ADP). They are postulated to act by selectively and irreversibly inhibiting one of a family of membrane bound nucleotide receptors (the P2 receptors) on the platelet surface^[10–12], possibly through inhibition of adenylate cyclase^[13–15]. One of their net effects on the platelet is to prevent activation of the GP IIb/IIIa receptor, which represents the final common pathway for platelet aggregation.

Both ticlopidine and clopidogrel have been studied in clinical trials in patients with atherosclerosis. The usefulness of ticlopidine is, however, limited by its potential to cause severe neutropenia in about 1% of patients, which necessitates close monitoring of blood counts at regular intervals, at least during the first few weeks or months of therapy^[16,17]. Ticlopidine^[18–20] and (to a much lesser extent) clopidogrel^[21] have also very rarely been associated with thrombotic thrombocytopenic purpura. In contrast to clopidogrel, the full antiplatelet action of ticlopidine is delayed for several days after commencement of therapy, limiting the usefulness of this agent in acutely ill patients and those undergoing non-elective percutaneous coronary intervention with stent implantation. By contrast the full antiplatelet action of clopidogrel after a 300 mg bolus is evident after several hours, making it useful in both acute and chronic settings^[22].

Meta-analysis of thienopyridines in vascular disease

Thienopyridines vs placebo/control or aspirin

Both ticlopidine and clopidogrel have been compared in several randomized trials vs placebo or aspirin in a wide variety of patients with cardiovascular, peripheral

vascular and cerebrovascular disease (Table 1)^[16,23–27]. A meta-analysis from three major studies of ticlopidine vs placebo or control in patients with atherosclerotic disease (total n=2392 patients) demonstrated a 29% relative risk reduction in vascular events (OR 0.71, 95% CI 0.58–0.86, $P=0.0006$) (Table 1). In two published studies of thienopyridine vs aspirin in patients with vascular disease (total n=22 254 patients), thienopyridines were more effective than aspirin in reducing the frequency of major ischaemic events (OR 0.90, 95% CI 0.83–0.97, $P=0.009$) (Table 1). The largest of these trials was the CAPRIE study which randomized 19 185 patients with previous myocardial infarction, recent transient ischaemic attack/stroke or symptomatic peripheral vascular disease to clopidogrel 75 mg a day or aspirin for a period of 1–3 years. At a mean of 1.9 years of follow-up, clopidogrel had significantly reduced the primary outcome of vascular death, myocardial infarction or ischaemic stroke compared with aspirin by 8.7% (95% CI 0.3%–16.5%)^[27].

Thienopyridines plus aspirin vs aspirin alone or oral anticoagulation

Thienopyridines and aspirin act through complementary and independent mechanisms, and their combination can inhibit both ADP-induced platelet aggregation and thromboxane A₂ production^[28,29]. The superiority of this combination, compared with aspirin alone or with oral anticoagulation, has been demonstrated in several randomized trials in patients after coronary artery stenting^[30–34]. A meta-analysis of these trials demonstrates a marked benefit of aspirin plus ticlopidine in reducing death or myocardial infarction compared with aspirin alone (OR 0.23, 95% CI 0.11–0.49, $P=0.0001$) or with aspirin plus warfarin (OR 0.51, 95% CI 0.33–0.78, $P=0.002$) (Figs 1 and 2). Several studies have compared the combination of clopidogrel and aspirin or ticlopidine and aspirin after coronary stenting; these suggest that aspirin and clopidogrel is better tolerated and at least as safe and effective as aspirin and ticlopidine^[35–39]. Although combination therapy with a thienopyridine and aspirin appears to be superior to aspirin alone in patients undergoing stent implantation, its role in the much larger population of patients with acute coronary syndrome is unknown.

The Clopidogrel in Unstable Angina Recurrent Events (CURE) trial was designed to test the hypothesis that treatment with the combination of clopidogrel and aspirin is superior to aspirin alone when initiated early and continued long-term, in the prevention of major cardiovascular events in patients with acute coronary syndrome.

Methods

Study design

CURE is an international, multicentre, randomized, parallel group trial of the combination of clopidogrel

Table 1 Vascular death, myocardial infarction or stroke in major clinical trials of thienopyridines versus placebo or ASA in patients with vascular disease

Trial, Year	Setting	Thienopyridine (n/N)	Comparator (n/N)	Odds Ratio	95% CI
Thienopyridine versus placebo or control					
CATS, 1989 (Ticlopidine vs Placebo)	Recent stroke	108/525	134/528	0.74	0.56–0.99
Balsano 1990 (Ticlopidine vs Control)	Unstable Angina	23/314	48/338	0.49	0.30–0.81
STIMS, 1990 (Ticlopidine vs Placebo)	Intermittent claudication	91/346	108/341	0.77	0.55–1.07
Total*		220/1185	290/1207	0.71	0.58–0.86†
Thienopyridine versus ASA					
TASS, 1989 (Ticlopidine vs ASA)	Cerebral ischaemia	370/1529	395/1540	0.93	0.79–1.09
CAPRIE, 1996 (clopidogrel vs ASA)	Recent stroke, previous MI or PVD	939/9599	1021/9586	0.91	0.83–1.00
Total*		1309/11128	1416/11126	0.91	0.84–0.99†

*Combined odds ratio computed using the Peto–Yusuf modification of the Mantel–Haenszel method^[13,44].† $P=0.0006$.‡ $P=0.03$.

CATS: Canadian American Ticlopidine Study.

TASS: Ticlopidine Aspirin Stroke Study.

STIMS: Swedish Ticlopidine Multi-centre study.

CAPRIE: Clopidogrel versus Aspirin in Patients at Risk of Ischaemic Events.

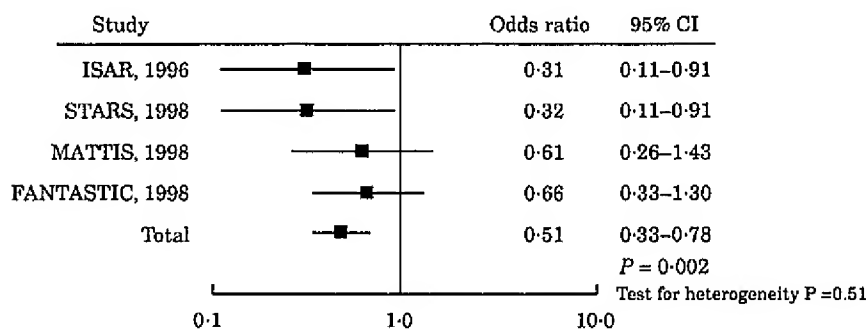


Figure 1 Death or myocardial infarction in trials of aspirin+ticlopidine vs aspirin+oral anticoagulation after coronary artery stenting. *STARS was a 3 arm trial. Data from aspirin+ticlopidine vs aspirin+oral anticoagulation arms were used in this analysis.

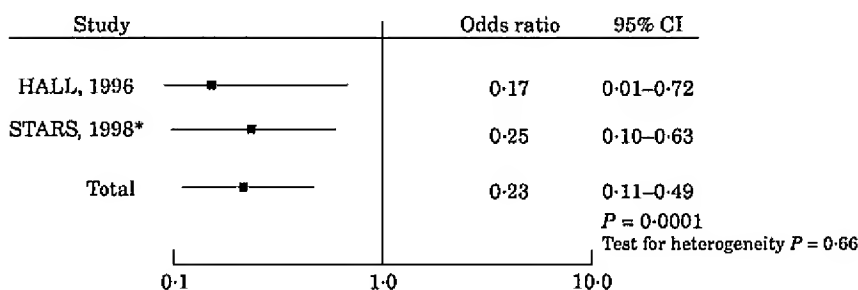


Figure 2 Death or myocardial infarction in trials of aspirin+ticlopidine vs aspirin alone after coronary artery stenting. *STARS was a 3 arm trial. Data for aspirin+ticlopidine vs aspirin alone arms were used for this analysis.

plus aspirin vs placebo plus aspirin in patients with acute coronary syndrome (unstable angina and non-Q wave myocardial infarction). The study involves 508 centres from 28 countries.

term need for oral anticoagulants and PTCA/stent or CABG within 3 months prior to randomization are excluded.

Study population

Patients are eligible for CURE if they are admitted to hospital with symptoms suggestive of an acute coronary syndrome without ST segment elevation greater than 1 mm, and presenting within 24 h of onset of the most recent episode of ischaemic chest pain/symptoms. Either ECG changes compatible with new ischaemia or already elevated cardiac enzymes or troponin I or T to at least twice the upper limit of normal is required for inclusion in the study.

Initially, patients above the age of 60 with no new ECG changes but with objective evidence of ischaemia were included in the trial. However, after a review of the overall event rates among the first 3000 patients enrolled in the trial, the steering committee recommended that all patients have either ECG changes or a cardiac enzyme rise at entry. Patients with contra-indications to antithrombotic/antiplatelet therapy, those at high risk of bleeding, severe heart failure (class IV), ongoing long

Randomization

Patients are randomized to either clopidogrel or placebo in CURE by a telephone call to a central, 24-h, computerized randomization service located at the Canadian Cardiovascular Collaboration Project Office, McMaster University, Hamilton, Canada. Permuted block randomization, stratified by clinical centre is used.

Treatment protocol

A loading dose of clopidogrel 300 mg orally or matching placebo is administered as soon as treatment is allocated (Day 1). Aspirin (recommended dose 75-325 mg daily) is started (or continued if patients were already taking aspirin prior to study enrolment) simultaneously with the blinded study drug. Treatment will continue (clopidogrel 75 mg per day or matching placebo) for a

Table 2 Minimum relative risk reduction detectable with a total sample size of 12 500 patients (6250 per group)

Event rate in placebo arm	Alpha* (two sided)	Detectable relative risk reduction	
		With 80% power	With 90% power
CV death, MI, stroke			
10%	0.045	14.7%	16.9%
12%	0.045	13.3%	15.3%
CV death, MI, stroke, refractory ischaemia			
14%	0.01	14.6%	16.4%
16%	0.01	13.6%	15.3%

*Type 1 error.

CV=cardiovascular; MI=myocardial infarction.

minimum of 3 months (for the last patient recruited) or for varying periods up to a maximum of 1 year for the remaining patients.

Follow-up

Follow-up assessments will occur at baseline, hospital discharge, and at 1 month and 3 months (with additional follow-up visits at 6, 9 and 12 months for those patients randomized early in the study).

Study outcomes

The co-primary outcomes in CURE are: (1) the composite of cardiovascular death, myocardial infarction or stroke and (2) the composite of cardiovascular death, myocardial infarction, stroke or refractory ischaemia. Events over the duration of follow-up will be included.

Sample size

The initial sample size of CURE was 9000 patients. Assuming a range of control event rates between 12% and 14% for cardiovascular death, myocardial infarction or stroke, the power of the sample size was estimated to be 80% to detect a 14.1% to 15.4% relative risk reduction; or 90% to detect a relative risk reduction of 16.2% to 17.7% ($2\alpha=0.05$). However, after review of the blinded overall event rates when 5000 patients had been randomized, there was concern that the projected event rates at 1 year may be significantly lower. Therefore, the study size was increased to include 12 500 patients, and co-primary outcomes were adopted. It is expected that a study of this size will result in about 1250 to 1500 events. Assuming control event rates of 10% to 12% for the outcome of cardiovascular death, myocardial infarction and stroke and a two-sided α of 0.045 for this outcome, the power of such a study will be 90% to detect a 15.3% to 16.9% relative risk reduction in this composite (Table

2). For the co-primary outcome of cardiovascular death, myocardial infarction, stroke or refractory ischaemia, assuming control event rates of 14% to 16% and a two-sided α of 0.01, the power to detect relative risk reductions of 15.3% to 16.4% will be 90% in this composite (Table 2).

Data analysis

The primary analysis in CURE will be based on the intention-to-treat approach. It will compare the first occurrence of an event in the co-primary composite outcomes of A: cardiovascular death, myocardial infarction or stroke or B: cardiovascular death, myocardial infarction, stroke or refractory ischaemia over the average duration of follow-up, using the log-rank statistic and maintaining an overall two-sided α of 0.05. Taking into account correlation between composites A and B, and given that composite A represents approximately 55% of events in composite B (i.e. cardiovascular death, myocardial infarction or stroke is a subset of cardiovascular death, myocardial infarction, stroke or refractory ischaemia), clopidogrel would be considered superior to placebo if the difference in composite A is significant at $P<0.045$ or composite B is significant at $P<0.01$.

Interim analyses

The independent Data and Safety Monitoring Board (DSMB) will monitor the progress of all aspects of the study and ensure that the study meets the highest standards of ethics and patient safety. For efficacy, the co-primary outcomes will be monitored using a modified Haybittle-Peto boundary of four standard deviations in the first half of the study and three standard deviations in the second half of the study. The boundary must be exceeded on at least two consecutive time points, 3 months apart. Futility will be assessed by the B-value (the conditional power for reaching significance by the end of the trial, given the current trends in the data), which will be calculated along with the 95% confidence

Table 3 Baseline demographics and past medical history and electrocardiographic changes in all randomized patients (N=12563)

Age [years, mean (\pm SD)]	64.2 (11.3)
Gender (% F)	38.5%
Time from onset of CP to randomization [hours, mean (\pm SD)]	14.1 (7.1)
Heart rate [beats/minute, mean (\pm SD)]	73.1 (14.8)
Systolic blood pressure [mmHg, mean (\pm SD)]	134.3 (22.3)
Diagnosis at entry	
Unstable angina	74.8%
Suspected myocardial infarction without ST elevation	25.0%
History of	
Myocardial infarction	32.0%
Coronary artery bypass graft surgery	11.0%
Percutaneous coronary intervention	9.8%
Stroke	4.0%
Heart failure	7.5%
Hypertension	58.5%
Diabetes mellitus	22.5%
Current smoker	23.0%
Former smoker	37.7%
ECG abnormality	
ST depression >1 mm	41.9%
Major T wave inversion >2 mm deep	25.6%
Other T wave inversion <2 mm deep	11.3%
ST elevation <1 mm	3.2%
Transient ST elevation >2 mm	0.6%
Abnormal ECG	93.4%

interval. If the upper limit of the confidence interval is less than a conditional power of 25% (faint hope), then all other things being equal, the DSMB may recommend early termination. There will be two formal interim assessments performed by the DSMB-associated statistician to assess efficacy, scheduled at approximately 1/3 and 2/3 of expected events. Safety aspects, and more specifically life-threatening bleeding, will also be monitored. No formal boundaries for assessing safety will be proposed, but clear, consistent, and persistent evidence of net harm that overwhelms any benefit could be the basis to discontinue the study.

Central adjudication

A central committee of clinicians who are blinded to treatment allocation will adjudicate the following outcomes: cardiovascular death, new myocardial infarction, stroke, refractory ischaemia, major and life-threatening bleeding.

Baseline characteristics

A total of 12563 patients were randomized into cure. Baseline demographics, electrocardiographic abnormalities and medication use in all randomized patients are shown in Tables 3 and 4. These data show that the mean age is 64.2 years, 38.5% of patients are women, 22% of patients have previously diagnosed diabetes and over 32% have had prior myocardial infarctions. In addition,

Table 4 Medications at time of randomization and during initial hospitalization (N=12563)

	At randomization	In-hospital
Aspirin	66.5%	99.1%
Heparin	37.9%	46.0%
Low molecular weight heparin	32.2%	54.0%
IV glycoprotein IIb/IIIa inhibitors	0.1%	3.2%
ACE inhibitors	36.0%	48.9%
Beta-blockers	57.5%	77.5%
Calcium channel blockers	27.8%	35.0%
Lipid lowering agents	24.9%	45.6%
Intravenous nitrates	44.4%	52.9%

over 92% of patients in the trial have an abnormal ECG, with the most common abnormality being major ST depression (41.5%). These data are consistent with at least a moderate risk group of patients with acute coronary syndrome. Medication use in-hospital reveals high in-hospital rates of use of aspirin (>99%), heparin (46% unfractionated heparin, 54% low molecular weight heparin) and beta-blockers (>77%).

Unique aspects and substudies of CURE

(A) *Epidemiological assessments (EPI-CURE)* EPI-CURE is a large epidemiological study, which has been integrated into the main CURE trial. The principal objective of EPI-CURE is to better understand the genetic and pathophysiological mechanisms and risk factors for patients with acute coronary syndromes. It

uses both a cohort design (for non-ST elevation acute coronary syndrome) made up of patients enrolled into CURE and a case-control approach which includes two types of cases: those with unstable angina, and new incident cases of acute transmural myocardial infarction. These cases are matched to age and gender control subjects. Factors to be analysed include blood counts, markers of coagulation, lipids, markers of inflammation, insulin, fructosamine and genetic markers of a large variety of candidate genes for atherosclerosis and its intermediate phenotypes. This substudy will generate and integrate substantial biochemical and genetic data in patients with acute coronary syndromes with their clinical presentation and course. These data will also be used to explore the impact of clopidogrel in various subsets of patients as defined by their baseline levels of different biochemical and genetic markers.

(B) Assessment in patients undergoing percutaneous coronary intervention (PCI-CURE) PCI-CURE is a percutaneous coronary intervention substudy designed to evaluate outcomes in patients enrolled in the CURE study who undergo percutaneous coronary intervention. Specifically, it will assess whether patients who are pre-treated with clopidogrel prior to percutaneous coronary intervention have superior outcomes compared with those not pre-treated^[40,41]. The hypothesis is that optimal antiplatelet coverage with the combination of clopidogrel and aspirin started before percutaneous coronary intervention will be superior to aspirin alone in preventing major cardiovascular events. The primary outcome will be the composite of death, myocardial infarction or urgent target vessel revascularization at 30 days. PCI-CURE will also assess whether longer term treatment (3 months to 1 year) with clopidogrel in patients undergoing percutaneous coronary intervention is superior to shorter term (less than 30 day) treatment in preventing the composite of cardiovascular death, myocardial infarction or refractory ischaemia. It is expected that over 2500 patients will undergo percutaneous coronary intervention while on therapy with blinded study medication.

(C) Assessment of coagulation data (COAG-CURE) COAG-CURE is a coagulation marker substudy designed to assess whether treatment with the combination of clopidogrel and aspirin is superior to aspirin alone in suppressing markers of thrombin generation and activity, fibrinolytic activity and platelet activation.

(D) Millennium survey This survey will collect data on practice patterns in patients with acute coronary syndromes at the turn of the millennium. It will include all consecutive patients presenting to the centre with acute coronary syndrome (not only those enrolled in CURE) during a 4-week period. Data to be collected include use of cardiac procedures, in-hospital medical therapy, and major in-hospital cardiovascular outcomes (myocardial infarction, stroke, and death). It will provide a unique 'snapshot' of practice patterns in 28 countries

for acute coronary syndrome at the turn of the new millennium.

Summary

A meta-analysis of randomized trials of thienopyridines in patients with vascular disease demonstrate that thienopyridines reduce vascular events by 27% when compared with placebo/control, and by 10% when compared with aspirin. Similarly, in patients undergoing intracoronary stent implantation, thienopyridines used in combination with aspirin reduce death or myocardial infarction by 77% when compared with aspirin alone and 49% when compared with aspirin plus oral anti-coagulation. The CURE trial is a large international study designed to determine if acute and long-term treatment with the combination of clopidogrel and aspirin is superior to aspirin alone in patients with acute coronary syndrome. Baseline characteristics reveal a study population that is consistent with at least a moderate risk group of patients with acute coronary syndrome.

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Appendix

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